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METHODS OF STAINING TUBERCLE BACILLI*

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IN choosing a laboratory test for practical purposes the clinical pathologist usually weighs speed, efficiency and reliability in his choice. There is no obvious reason why this policy should be deviated from in choosing a method for staining tubercle bacilli in suspected materials from human sources for diagnostic or prognostic purposes.

It is of historical interest that Koch[1] in 1882 stained the bacilli by means of methylene-blue (1 c.c. concentrated alcoholic in 200 c.c. distilled water + 0.2 c.c. of 10 per cent potassium hydroxide), using freshly filtered concentrated watery vesuvin solution as a counterstain, thus obtaining blue bacilli on a brown field, while Baumgarten[2] at the same time studied them unstained by contrast in a stained field. Koch early recognized the resistance of the bacilli to staining as well as destaining and utilized a small amount of potassium hydroxide in the methylene-blue stain for this reason to aid penetration. Originally he used no decolorizing agent but after staining for long periods at room temperature, or shorter periods with warming, applied the counterstain vesuvin. The shortcomings of the Koch method were early recognized and Ehrlich[3] introduced aniline oil into the staining technic with decided improvement. The Ehrlich method consisted essentially in staining the smear or section (twelve hours cold or less time warm) in a solution containing 11 c.c. of concentrated alcoholic methyl-violet or fuchsin in 100 c.c. of saturated aniline oil water and 10 c.c. absolute alcohol, decolorizing in dilute (1:3) nitric acid for a few seconds, rinsing in 60 per cent alcohol and counterstaining in dilute

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vesuvin. It is interesting to note that Ehrlich stained the bacilli with a large number of basic stains including vesuvin or Bismarck brown.

There rapidly followed an elaborate literature and numerous new methods for staining tubercle bacilli, each claiming advantages and adding valuable information to the sum total knowledge on this subject. Of practical importance, however, were the introduction of carbolic acid solution of the basic stains by Ziehl[4] and the use of carbol diamant-fuchsin by Neelsen[5] constituting the rapid Ziehl-Neelsen method now so widely used routinely for staining tubercle bacilli.

In order to still further simplify the technic Gabbett,[6] on the basis of a recommendation made by B. Fränkel earlier, combined the decolorizing and counterstaining by using a 25 per cent watery solution of sulphuric acid containing 1 to 2 grams of methylene-blue in 100 c.c. of solution. From the time Koch found potassium hydroxide aided penetration of the bacilli by the stain and Ehrlich used aniline oil for this purpose there resulted a large number of methods based on the use of different mordants including, besides carbolic acid, resorcin, pyrogallic acid, ammonium carbonate, ammonium hydroxide, boric acid, chloroform, creosote, menthol, mercuric chloride, and others. So, likewise, there have been recommended a large list of decolorizing agents including acetic, hydrochloric, nitric and sulphuric acids, acid alcohol, lactic acid, acetone, sodium sulphite, hydrogen peroxide, calcium hydrochlorite and even hot water.

Since it was practically impossible to test out all the methods that have been recommended for staining tubercle bacilli, only those will be referred to here which were considered of sufficient importance to be included in a comparative test when this study was initiated. They were chosen for a number of reasons among which were that they were recommended from a number of sources in the literature; the method served basically for the origin of a number of later methods and it incorporated essentially a new principle founded on seeming sound ideas.

Among the older methods in which a chemical mordant was emphasized was that described by Herman[7] who used for this purpose ammonium carbonate. The stain consists of 3 parts of 1 per cent ammonium carbonate solution in distilled water and 1 part of 3 per cent crystal violet in 95 per cent methyl alcohol. The smear or section is stained for a few minutes steaming and is then decolorized with 5 or 10 per cent nitric acid solution for a few seconds after which it is dipped a few times in 95 per cent alcohol and then is rinsed in tap water and is counterstained with 1 per cent eosin solution (originally he used 60 per cent alcohol as solvent). The bacilli appear violet in a rose field. In 1908 the author claimed to find bacilli when Ziehl-Neelsen's method failed and that it was superior to Much's method.

Another of the earlier methods was that of Andrejew[8] who not only utilized the mordanting idea, using for this purpose potassium chloride, but laid stress upon complementary counterstaining. It is pointed out that green is the most suitable counterstain for red bacilli and of the available green stains he prefers acid green to malachite green. The preparations are stained by the ordinary carbol-fuchsin solution and are decolorized and counterstained

for about one minute in a mixture of (1) hot 10 per cent potassium chloride solution 100 c.c.; (2) acid green 1 gram; and (3) 25 per cent sulphuric acid 15 c.c.; the mixture having been well shaken and filtered.

Among the serviceable yet uniquely developed methods for staining tubercle bacilli may be included that described by C. Spengler,[9] and devised to fit the theory that tuberculosis in man usually was a double infection with human and bovine bacilli which could be differentiated by his staining methods. He also believed that the presence of granules, called by him "Splitter" or "sporoide Körper" demonstrated by one of the three methods devised by him, was of diagnostic value. Later he considered them spores, analogous to the spores of anthrax. The picrin method of Spengler essentially consists of staining with warm carbolfuchsin for five minutes, which is then poured off, and picric acid-alcohol (50 c.c. saturated watery picric acid + 50 c.c. absolute alcohol) applied for two to three seconds, followed by three to four drops of 15 per cent nitric acid and then by picric acid 5 to 10 seconds until the sputum takes on a faint yellow color after which the smear is rinsed in water and dried; or after picric acid treatment the smear is rinsed with 60 per cent alcohol followed by 15 per cent nitric acid (a few seconds) until the sputum assumes a light yellow color, when it is rinsed with alcohol, followed by counterstaining with picric acid alcohol to a yellow color, after which the smear is rinsed in water, dried and examined. It is claimed that the method gives positive results when the Ziehl-Neelsen and other methods fail and that it is specific for acid-fast organisms. The light red bacilli and isolated "splitter" are distinctly seen in the light yellow background.

Until Much[10] emphasized the importance of Gram staining the granules of tubercle bacilli the attention had been focused mainly on the importance of mordanting. Much's method consisted of 3 distinct technics all based on the use of iodides such as are used in making the ordinary Gram stain.

Much's technics were as follows:

Gram method I.

1. Aniline water-gentian violet;
2. Lugol solution;
3. Decolorizing in absolute alcohol and clove oil.

Gram method II.

1. Methyl-violet B. N. 10 c.c. saturated alcoholic solution in 100 c.c. 2 per cent carbolic acid water. Steam over the flame three minutes or warm twenty-four to forty-eight hours at 37° C.
2. Iodpotassium iodide one to five minutes;
3. Five per cent nitric acid one minute;
4. Three per cent hydrochloric acid ten seconds;
5. Acetone-alcohol āā.

Gram method III.

1. Methyl-violet B. N. solution as above;
2. Potassium iodide-hydrogen peroxide solution (5 g. KI in 100 c.c. 2 per cent H_2O_2) for two minutes;
3. Absolute alcohol.

Much claimed that his method would reveal all the granular forms of tubercle bacilli not stained by the Ziehl-Neelsen's method and concludes that it seems to him that the Ziehl method stains substances in the tuberculosis virus which differs from that stained by Gram, and that the Gram method still gives

positive results when the Ziehl staining substances are lacking. In a polemic with Fuchs-Wolfring, Much[10a] also takes a definite stand regarding the relation of the Gram granules and Spengler's "Splitter." He says "the Much granules are not acid-fast. The Splitter are acid-fast (picrin method)."

Rapidly following this observation by Much there were reported a number of combination stains developed in an endeavor to combine the advantages of Gram staining and bacillary staining based upon either the Spengler or Ziehl-Neelsen methods. One of these was described by Fontes[11] who also believed his method would differentiate the true tubercle bacillus from pseudotubercle bacilli. The smears are stained in carbolfuchsin for one minute steaming and are then allowed to stand in contact with the stain two minutes longer when they are rinsed with water and stained for two minutes with steaming crystal violet or carbol gentian-violet after which the excess stain is removed and Lugol's solution allowed to act on the smear until no further metal mirror forms. The preparation is decolorized in acetone-alcohol (āā), is washed in water and counterstained with methylene-blue. It is claimed that the acetone-alcohol frees all the pseudotubercle bacilli from fuchsin. The violet granules appear very contrasty in the red bacillary bodies. Pseudotubercle bacilli are violet and have deeper violet granules while other gram-positive microorganisms (streptococci, staphylococci, etc.) are gram-positive and all other bacteria are blue. If the staining with fuchsin and crystal violet is reversed the same results attain but the picture is less clear. The granules vary from 1 to 6 in each bacillus although rarely there may be 8 to 10. Fontes concludes the granular forms are the more resistant forms.

For the purpose of differentiating of smegma from tubercle bacilli in the urine Gasis[12] has taken advantage of the alkali fastness of the tubercle bacillus in devising a method. "Tubercle bacilli behave amphoterically to acids and alkalies. Smegma bacilli are partly acid and alcohol resistant. Smegma bacilli are not alkali resistant. Tubercle bacilli are partly acid and alcohol resistant. Tubercle bacilli are alkali resistant." The Gasis method based on the foregoing facts consists of staining the fixed preparation one to two minutes warm with the dye-mordant combination made by boiling a small lentil size lump of mercuric chloride in a few (5) cubic centimeters of 1 per cent eosin solution (1 g. eosin, 5 c.c. absolute alcohol and 95 c.c. distilled water), with constant shaking until the mercuric chloride is completely dissolved. The smear is then rinsed in water and decolorized by a solution consisting of 0.5 sodium hydroxide, 1.0 potassium iodide and 100 of 50 per cent alcohol, until the red color has disappeared and a white green color develops. The decolorizing solution is removed by means of absolute alcohol and the smear is rinsed in water and counterstained with methylene-blue (1.0 g. + 10 c.c. absolute alcohol + ½ c.c. hydrochloric acid + 90 c.c. distilled water) for 2 to 3 seconds after which it is rinsed in water and dried for examination. The bacilli are a beautiful light red in a blue field. Gasis believes the granules obtained with this method are not identical with those obtained by the Much-Gram stain. The Gasis granules are deeper stained and more resistant than the bacilli he claims.

Within recent years there have been described three new modifications of the older methods which seem to merit consideration. One of these devised by

Konrich in 1920[13] may be attributed directly to the economy of war conditions. When the price of alcohol soared in Europe Konrich remembered the decolorizing reducing action of sodium sulphite on fuchsin in the preparation of Endo's medium and believed this might be advantageously and economically substituted for decolorizing in the Ziehl-Neelsen method for staining tubercle bacilli. The fixed preparation is stained one-half to one minute with hot carbol fuchsin, rinsed in water and is then treated a few seconds with a fresh 10 per cent watery solution of sodium sulphite after which it is rinsed in water and counterstained with malachite green solution (50 c.c. saturated watery solution + 100 c.c. water). He claims that acid alcohol may decolorize the bacilli but sodium sulphite does not, since it cannot enter the bacilli to reduce the fuchsin in them. They remain stained even after 24 hours treatment with sodium sulphite. Malachite green is preferred on account of its color contrast to the red bacilli and because it does not stain the rest of the cellular elements too deeply.

Another more recent modification was proposed by Burke and Dunning[14] who incidentally included the staining of the tubercle bacillus with the method for staining spores. The method essentially is apparently one of making a combined carbolfuchsin-Gram stain of the bacilli even though it is stated that "carbolfuchsin under the most favorable conditions does not give a good Gram reaction." The smear is stained in steaming carbolfuchsin for two minutes or longer, is washed in water, and decolorized with acetone or iodine acetone (1 part Gram's iodine solution to 3 parts acetone, or a few crystals of iodine is added to acetone) until the smear is nearly colorless which requires a few seconds, and is counterstained with 10 per cent aqueous solution of a saturated alcoholic solution of methylene-blue for one to two minutes or a saturated aqueous solution of picric acid for from five to ten seconds. Smears of tuberculous sputum stained by this method showed as many acid-fast organisms as when stained by the Ziehl-Neelsen method.

The third of the more recent methods by Serkowski[15] utilizes the fact frequently referred to as an advantage of the Spengler method—the possibility of staining the bacilli in thick smears without material interference with their visibility. The method may be considered a combination staining method in that 2 dyes, carbolfuchsin and carbol-methyl violet, are used with sodium carbonate as the mordant. (1) Liquid sputum is laid on a slide in several consecutive layers, each layer being allowed to dry before applying the next one. As the background remains practically unstained by this method, the thickness will not mask the bacilli; (2) cover film with carbolfuchsin without previous fixation and hold over flame two minutes during which steam must appear several times. (3) Without washing the film a 1 per cent solution of crystallized sodium carbonate is applied which is boiled over a flame two minutes, during which it may be necessary to add fresh sodium carbonate solution. The film must not be washed in water, but the excess of sodium carbonate is simply shaken off. (4) Stain with one part carbolfuchsin and three parts of carbol-methyl-violet (ten parts saturated alcoholic solution of methyl-violet and ninety parts 2 per cent carbolic acid in water) over flame for two minutes, steaming without ebullition. (5) Shake off excess stain, without washing in water, and

treat five minutes with 10 per cent solution of sodium sulphite (Konrich) adding a few drops of 60 per cent alcohol during the last stages of the decolorizing procedure. (6) Without washing, counterstain with 1 per cent picric acid for thirty seconds. (7) Remove all stain spots from the edges and back of slide with a cotton wool swab saturated in alcohol and acetic acid. (8) Then only is the slide dipped once or twice in still water and dried in air.

Serkowski claims this method increases the number of bacilli on the films 50 to 60 times over the Ziehl-Neelsen method and believes homogenization methods make the cortices of the bacilli less resistant to staining. Thistlethwaite[16] used this method and found on an average 8 bacilli to 1 in Ziehl-Neelsen preparations. She uses two or three layers of sputum fixed by heat before staining and prefers 10 per cent sulphuric acid for decolorizing.

In an attempt to accentuate the staining of tubercle bacilli Biot[17] after staining with carbolfuchsin and decolorizing with nitric acid places the preparation in commercial formalin for two to four minutes when, it is stated, the bacilli stand out as black rods. No counterstain is necessary by this method.

Fat stains have never been used with success for staining tubercle bacilli even though Dorset[18] at one time recommended certain of them (Sudan III and Scarlet R) for this purpose.

The opinions expressed and conclusions drawn from a study of a number of different staining methods for tubercle bacilli by some of the foremost students of this subject is worthy of brief mention here. Joest[19] in 1908-9 states as a result of experiences with the Much and Ziehl-Neelsen staining methods on tuberculous material that "the Gram (Much) staining for the bacterioscopic determination of the tuberculosis virus in lesions of spontaneous tuberculosis in cattle and pigs possesses no advantages over the Ziehl staining." Eisenberg[20] expresses himself in agreement with Liebermeister that in so far as diagnostic significance is concerned only rows of granules, and then only in otherwise bacterial free materials, are of value; dust like or free granules even though interesting theoretically are too little elucidated and too manifold in origin to be of any serious weight for diagnosis in practice.

As a result of extensive study with a large variety of tuberculous materials from animals and man using the Ziehl-Neelsen, Much and Gasis methods of staining tubercle bacilli Berger[21] in 1910 concluded that the Ziehl-Neelsen method is still the most reliable method, being negative only in a small number of cases positive by other methods, and it has the advantage of being rapidly and easily performed. It is especially reliable in cases of mixed infection where the Gram stain colors other rods. The disadvantage is that the granular forms are not stained by this method. The preparations by this method are also not permanent—the bacilli gradually becoming paler. The Much modified Gram method stains isolated and rows of granules, homogeneously stained rods are only rarely seen. This method is of great significance because it stains the granular forms of tubercle bacilli. The disadvantages of this method are the dangers of error in mixed infections and the entire picture is not as clear as after Ziehl-Neelsen staining. For rapid staining and speedy location of the bacilli the Gram method is less suitable than the Ziehl-Neelsen. It is not suited for permanent preparations. The Gasis method is especially suited for

structural studies of the tubercle bacillus. The disadvantages in comparison to the Ziehl-Neelsen method are its complicated nature and the greater percentage of failures in staining.

In an inaugural dissertation presented in 1910 Adam[22] tested a number of methods [Ziehl, Spengler, Gram (Much), Gasis, Berka, Knoll (a combination stain) and others] on a variety of tuberculous materials from man, cows, pigs, horses and chickens, and concludes that the picrin method of Spengler is of equal value to the Ziehl method. It has advantages over the Ziehl method when methylene-blue is used in the latter as counterstain. The Herman method, especially the Berka modification, is recommended as an adjunct to the Ziehl method. The Gram method of Much reveals a special form of tubercle bacillus not visible by the Ziehl method, but this method alone is not sufficient to certify a diagnosis of tuberculosis. The method of Knoll beautifully reveals the Much and Ziehl forms of tubercle bacilli but is only suited for a study of the finer structures of the tubercle bacillus. The method of Gasis is useful in differentiating tubercle bacilli from other acid-fast rods and for structural studies, but cannot replace the Ziehl method. None of the staining methods studied served to differentiate the types of bacilli.

In another inaugural dissertation presented in 1911 Barnowsky[23] tested sputum and other tuberculous material from man and animals, with and without antiformin treatment, by staining it according to the Ziehl-Neelsen and Much staining technics. He concludes that the Ziehl method stains tubercle bacilli homogeneously, rarely granular, while the Much method stains them granular, but rarely homogeneously. The granules occur in rod forms, occasionally in dust form. For diagnosis only the rod forms not the isolated granules can be evaluated since the latter have no definite form or size, and therefore cannot be differentiated from cocci, dye precipitates and other contaminations. Both methods fail to give permanent preparations; Much preparations last only twenty-four hours. Much's method after antiformin treatment must be cautiously interpreted since Gram positive rods and cocci can be mistaken for tubercle bacilli. Since the Ziehl technic is much more rapid and simpler than the Much technic, the former is given the preference. In suspected cases the Much technic can be utilized but in such cases animal inoculation must be relied upon to avoid mistakes in diagnosis.

Dold[24] in reporting on the differential diagnostic significance of various staining methods in 1911 states that there is no specific staining method available for the differentiation of the genuine tubercle bacillus from nonpathogenic acid-fast organisms and that comparative tests of the Ziehl and Gram (Much) methods on tuberculous tissues (lymph glands and spleen of guinea pigs) revealed no significant difference in the number of bacilli by these two methods. He does not wish to dispute the existence of non-Ziehl or non-acid-fast staining forms of tubercle bacilli but believes the diagnostic value of Gram positive rods or granules is slight. The double staining methods give instructive pictures and the Herman method is simple and serviceable. In 1911 Böhm[25] reported on trying 24 different staining methods for tubercle bacilli and concludes that "In the examination of suspected sputums the best results can be obtained with the Ziehl-Neelsen method since a bacillus found by this method

certifies the diagnosis. If the Much method is used and only granules are found a definite diagnosis cannot be made. Other methods do not come into consideration since they are partly less reliable and partly more complicated than the Ziehl method. On a par with the Ziehl method may be included the Ehrlich, Spengler and Herman methods.''

In a recent article Geschke[26] compares the Spengler method and one of its modifications, the Jötten-Haarmann, with the Ziehl-Neelsen method and two of its recent modifications, the Konrich and Schulte-Tigges methods. Of these five methods the Ziehl-Neelsen and Konrich with blue and green background proved the more pleasing to the eye but the bacilli are more distinct with the yellow counterstain. The Spengler and Jötten-Haarmann stained preparations of 56 sputums studied revealed far more bacilli than by the three other methods.

The study to be reported here was initiated for the purpose of helping elucidate the practical problem of staining tubercle bacilli for diagnostic purposes, and in addition to note where discrepancies in the practical methods were occurring with a view to correcting these and if necessary instituting a more standard and reliable technic than at present available. In order to obtain as much information as possible on the various better known staining methods for tubercle bacilli and especially those having initiated a new staining principle, 20 sputums positive for tubercle bacilli were stained by twelve different technics incorporating in some cases the ordinary principles of staining with a mordant as exemplified by the Ziehl-Neelsen method, in others the Splengler principle, and in some the Gram (Much) principle, or a combination of the latter with one of the former two. The methods used were the original Koch methylene blue-vesuvin method, Ehrlich's aniline oil-gentian violet method, Ziehl-Neelsen's carbolfuchsin-methylene blue method, Andrejew's fuchsin-acid green method, Spengler's carbolfuchsin-picric acid method, Much's methyl-violet B.N.—Gram method, Gasis' eosin mercuric chloride-methylene blue method, Herman's crystal violet-eosin method, Fontes' combination carbolfuchsin-Gram method, Konrich's modified carbolfuchsin-sodium sulphite method, Burke and Dunning's carbolfuchsin iodine method, and Serkowski's combination carbolfuchsin-carbolmethylviolet method. About three months were spent in trying out the various above methods in preparation for the staining of the twenty sputums so that all the methods might be given an equal chance in the final test on the sputums. In order to make the different smears from the same sputum as nearly alike as possible the sputum was well shaken with glass beads, filtered through cheesecloth, a portion thus well mixed placed on one glass micro-slide and two smears made by placing another slide on this one and drawing them apart. Every precaution was taken to have the smears from the same sputum alike in thickness and uniformity. Any that appeared to deviate from this even in general appearance was discarded. After the usual heat fixation all the smears of the 20 sputums to be stained by a certain method were stained at the same time and with as nearly as possible the same technic. After staining they were examined and the bacilli counted in an average of 100 high power oil immersion fields using a mechanical stage to obviate counting the same field twice and to permit a systematic examina-

tion of the smear. The results in number of bacilli found in the average field for each preparation are given for ten sputums only since they suffice to illustrate the significant findings. The results are given in Table I.

TABLE I

THE RESULTS OF THE EXAMINATION OF POSITIVE SPUTUMS FOR TUBERCLE BACILLI STAINED BY DIFFERENT TECHNICS

STAINING METHOD*	SPUTUMS									
	1	2	3	4	5	6	7	8	9	10
Koch	–	–	3.4	0.17	–	5.9	–	1.2	–	2.2
Ehrlich	3.2**	8.5	5.3	7.5	6.8	17.2	11.0	–	4.6	7.3
Ziehl-Neelsen	3.1	8.2	17.9	10.9	10.8	15.0	4.1	1.3	2.7	8.2
Andrejew	2.9	9.1	7.8	10.0	11.2	14.1	2.5	–	0.5	6.3
Spengler	3.2	10.0	3.0	7.0	8.1	14.1	6.1	1.5	1.7	5.0
Much	3.5	8.3	6.2	5.1	6.7	11.3	5.8	2.0	2.6	3.7
Gasis	–	4.8	5.9	–	2.4	–	4.4	–	–	5.2
Herman	3.3	14.7	14.0	7.5	4.6	4.87	3.1	–	0.4	10.0
Fontes	3.0	9.5	4.1	6.7	2.5	16.9	4.5	2.6	1.3	3.5
Konrich	3.5	15.6	14.2	12.3	15.3	20.0	10.2	1.5	2.2	4.9
Burke & Dunning	3.1	22.2	10.6	12.2	9.4	12.2	–	2.1	–	7.0
Serkowski	–	12.8	4.7	15.3	9.5	6.1	4.1	0.8	1.8	3.8

*Biot's method is not included in the tabulation although the sputums were stained by this method and examined, since it is only an intensified carbolfuchsin method which is more difficult of examination because of lack of counterstain, and the method seems to gain nothing at the expense of more cumbersome technic.

**The numerals indicate the number of bacilli per field obtained as an average from the examination of about 100 different fields. A dash indicates no bacilli were found stained in the preparation.

An examination of Table I reveals two outstanding points; first, that in spite of the fact that every precaution was used in staining the preparations, there were some smears in which the bacilli were not stained and this was not confined to any one technic but occurred irregularly with different methods, in addition to which there were at times obtained low counts seemingly attributable only to a poorer staining and, second, in spite of the precautions taken to obtain uniform smears these varied to the extent of permitting a difference of 100 per cent in the counts of individual smears. Some staining technics proved more difficult of performance as is evidenced by the greater number of failures. Among these were Koch's original method, Gasis and Biot's methods. A few methods proved unsatisfactory because of unsuitable staining of the bacilli, lack of a background counterstain or lack of contrast between the bacilli and remainder of the smear. Among these were Biot's and Serkowski's methods. Much's method proved entirely unsatisfactory with sputum on account of the difficulty encountered in differentiating the tubercle bacilli from other small cocci since only the beads or granules in the majority of cases stained by this method.

In order to exclude the variations consequent to the making of different smears from the same sputum, 16 different positive sputum smears were consecutively stained, decolorized and restained by nine different methods—Ziehl-Neelsen, Andrejew, Herman, Konrich, Gasis, Fontes, Burke and Dunning, Ehrlich, Spengler, and finally Ziehl-Neelsen again. The tubercle bacilli in an average of about 100 fields were counted on each smear. The findings for ten of sixteen of these sputum smears are recorded in Table II.

TABLE II

THE CONSECUTIVE RESTAINING OF POSITIVE SPUTUM PREPARATIONS FOR TUBERCLE BACILLI BY DIFFERENT STAINING TECHNICS

STAINING TECHNICS	SPUTUMS									
	1	2	3	4	5	6	7	8	9	10
Ziehl-Neelsen	6.2*	9.1	7.5	34.0	22.3	9.6	17.7	5.2	31.3	9.7
Andrejew	4.6	9.2	7.7	39.2	–	7.3	18.4	5.4	26.5	–
Herman	4.9	10.7	6.6	28.1	11.5	10.6	20.5	7.6	27.8	10.9
Konrich	3.4	10.7	4.4	28.2	32.2	11.2	23.4	6.9	24.6	11.0
Gasis	1.3	4.5	1.5	8.6	–	12.7	19.8	6.0	10.4	2.1
Fontes	4.9	7.6	7.0	25.3	19.7	8.0	19.0	5.0	25.9	8.9
Burke & Dunning	4.5	9.3	3.3	26.6	16.9	10.9	19.0	6.7	28.3	12.3
Ehrlich	3.3	11.8	4.7	23.1	10.2	9.6	14.5	5.0	25.3	10.6
Spengler	3.2	3.2	–	26.4	15.2	10.4	15.3	4.6	21.2	5.8
Ziehl-Neelsen	5.8	10.5	6.9	24.1	23.1	10.1	23.6	6.8	37.6	9.7

*The numeral indicates the bacilli in an average field of about 100 counted; a dash indicating no stained bacilli were found.

Table II brings out clearly the importance of technic in the practical staining of tubercle bacilli rather than the importance of method which seems to have been emphasized mostly in the past. The choice of method resolves itself more into the question of getting thoroughly acquainted with one or two of them that happens to meet the fancy of the operator and by performing them accurately and carefully they will give results approximately equal to any of the other reliable methods. Of course, sight must not be lost of the fact that, given the choice of two equally efficient methods, the time element remains the important factor. This table also gives a good insight into the possible variations in counts resulting from slight possible deviations in technic. It will be noted in addition that a combination, double bacillary stain technic as exemplified in the Fontes method, neither increased the efficiency of the simple single bacillary stain methods nor did it obviate the deviations, consequent to slight variations in technic, of the single bacillary stain method.

In an effort to obtain more information on the granules staining by the carbolfuchsin simple mordant technic as exemplified in the Ziehl-Neelsen method, four positive sputum slides were stained gradually by means of steaming carbolfuchsin, each time being examined after decolorizing in 3 per cent acid alcohol and counterstaining with methylene-blue, and it was noted that in all four slides with faint bacillary staining there was a distinct granule staining present in the large majority of the tubercle bacilli in the smear. The granules were distinctly red when the bacillary body was a faint pink or was still unstained. As the stain intensity increased the granules became a dark red in contrast to the lighter red of the bacilli and finally with additional staining the entire bacilli assumed a dark purplish red uniformly stained appearance. Comparison of these carbolfuchsin stained slides during the predominant distinct coloring of the granules revealed them in numbers from 1 to as high as 6 or 8 in the individual tubercle bacilli and except for the deep red color of the granules as contrasted to the dark blue of the Gram (Fontes and Much) stained slides of the same sputums they seemed to be identical. The above four smears were then gradually decolorized by means of 3 per cent acid alcohol and after counterstaining with methylene-blue were repeatedly examined. Gradually the tubercle bacilli lost their intense dark reddish color

and the dark granules again appeared in a lighter red color of the bacilli themselves. As the decolorization proceeded both the granules and bacilli became lighter red but the granules were regularly darker than the remainder of the bacilli and finally there were in certain instances found granules still stained red with the rest of the bacillary body unstained and only seen as a transparent colorless shadow barely visible only in a few cases. These same four slides were then stained by the Gram and carbolfuchsin combined method and only the blue Gram granules could be found, there being no red granules, which fact would seem to speak in favor of the identity of the two types of granules. If the Gram granules and ordinary carbolfuchsin staining granules obtained during Ziehl-Neelsen staining differed it would seem that the combination stains at times at least might reveal both red and blue granules in a pale pink tubercle bacillus. It must be admitted that the blue Gram granules in the red bacillus are much more striking to the eye than the red granules but this concerns mainly the more striking color contrast of blue on red as compared to dark red on red or red on pink which also tends to make the granules look different both in size and morphology. The contention that the Gram granules are not acid-fast seems incorrect also, since Gram (Much) stained preparations may cautiously be stained with steaming carbolfuchsin and decolorized with 3 per cent acid alcohol removing the fuchsin from all parts of the smear except the bacilli, and blue granules are still visible in the red bacilli.

Thus far sputums containing besides tubercle bacilli numerous other microorganisms, were used as tests of the various staining methods and since the Gram modifications are supposed to possess advantages in the examination of pus and materials free from other microorganisms, a series of 12 samples of pus were stained by the Ziehl-Neelsen, Spengler, Much and Fontes methods, 100 fields being counted in each specimen and the findings recorded in Table III. The pus examined was obtained from guinea pigs inoculated subcutaneously, thirty to thirty-five days prior to obtaining the specimen, with avirulent ("Human") and virulent ("Gluckson") human and virulent ("Bov. Vir.") bovine tubercle bacilli. The pus was well ground up, first as obtained and then after the gradual drop by drop addition of sterile distilled water, until a fine

TABLE III

RESULTS OF THE EXAMINATION OF PUS, CONTAINING HUMAN OR BOVINE TUBERCLE BACILLI, STAINED BY THE CARBOLFUCHSIN (ZIEHL-NEELSEN AND SPENGLER) AND GRAM (MUCH AND FONTES) METHODS

STAINING METHOD	AMOUNT IN MILLIGRAMS AND TYPE OF BACILLI USED FOR INFECTING GUINEA PIGS SUBCUTANEOUSLY											
	"HUMAN" (AVIRULENT HUMAN BACILLI)				"GLUCKSON" (VIRULENT HUMAN BACILLI)				"BOV. VIR." (VIRULENT BOVINE BACILLI)			
	50 MG.	50 MG.	1.0 MG.	1.0 MG.	0.1 MG.	0.1 MG.	0.001 MG.	0.001 MG.	0.1 MG.	0.1 MG.	0.001 MG.	0.001 MG.
Ziehl-Neelsen	0.23*	0	0	0	0.17	0.19	0.09	0.02	0.18	0.12	0.27	0.05
Spengler	0.16	0	0.01	0.18	0.11	0.09	0.08	0	0.09	0.05	0.12	0.02
Much	0.06	0	0.01	0	0.19	0.02	0.03	0	0.10	0.03	0.15	0
Fontes	0.02	0	0	0	0	0.01	0	0	0.04	0	0.05	0

*At least 100 fields were examined in each preparation and the numeral given is the average number of bacilli per field deduced therefrom. The staining and counting of bacilli was performed by different individuals. The counting was checked by two individuals to obviate as far as possible individual error and in the Gram stains only bacillary forms were counted to avoid the possibility of including individual cocci or dye precipitates as tubercle bacilli. Any semblance of bacillus was, however, counted as such.

uniform suspension resulted. The smears were made as uniformly as possible and after heat fixation were stained.

An examination of Table III reveals no decided advantages of the Gram over the ordinary carbolfuchsin methods although it must be admitted, even though the reverse appears evident from the table, that as a method, except for more complicated technic involved, the combined method (Fontes) properly performed probably gives equally good results to the simple carbolfuchsin methods. The simple Gram Much technic is always fraught with the danger of misinterpretation for practical purposes. The variable figures recorded in Table III again emphasize the difficulty of making absolutely uniform smear preparations and the importance of proper staining technic. It is for this reason that it seems advisable especially that the clinical pathologist, who is generally called upon to examine different materials of which he can frequently obtain only a single sample and who has manifold methods for different diagnostic purposes with which he must keep efficiently conversant, have available a technic that will rule out or reduce it to a minimum. In the tuberculosis sanatorium or hospital where repeated specimens can be obtained this may not be so essential but even there it would seem advisable in examining certain specimens such as urine, pus and spinal or pleural fluid. It is a well-known fact that tubercle bacilli retain their staining properties for years and that even boiling or autoclaving and certain drastic chemical treatments will not materially affect this. It is an easy matter, therefore, for every clinical pathologist to keep at hand a standard suspension of tubercle bacilli which he can appropriately mix with a sample of the specimen to be tested—urine sediment, spinal fluid, pus, etc.—and making a control smear with this can carry it through the identical technic as that used on the test smear. This will give him confidence in his technic applied to the particular material at hand being tested. If his technic is correct the bacilli in the control preparation should stand out well stained and in number equivalent to his accustomed observations with the same suspension. On account of the difference of staining and destaining of thick and thin portions of smears it also seems advisable to make control and test smears as nearly uniform as possible.

In tests of various mixtures for this purpose it has been found that a suspension of tubercle bacilli made by carefully grinding tubercle bacilli with 0.9 per cent sodium chloride solution and finally diluted to contain 1 to 10 milligrams of tubercle bacilli per cubic centimeter in 50 per cent acetone-saline solution, mixed with sputum, pus or urine in proportion of approximately 0.1 c.c. of suspension to 0.9 c.c. of material to be tested, gives sufficient bacilli (5 to 10) in the ordinary oil immersion field to yield a speedily examined check on the staining technic. The acetone-saline suspension of tubercle bacilli keeps well in a rubber-stoppered bottle and on shaking gives a good uniform suspension each time. Suspensions made with eggwhite, gum acacia and glycerol did not prove satisfactory. The one danger in the use of this—control method—is that of contamination of the test specimen under examination, which would lead to erroneous positive findings. The careful worker need

not be warned of this danger, yet it must be kept in mind where the work of testing specimens is relegated to careless or untrained technicians.*

SUMMARY AND CONCLUSIONS

An examination of 20 sputum smears containing tubercle bacilli by thirteen different staining methods (Koch, Ehrlich, Ziehl-Neelsen, Andrejew, Spengler, Much, Gasis, Herman, Fontes, Konrich, Burke and Dunning, Serkowski, and Biot) revealed that when the technic is properly performed the majority of these methods may result in staining the tubercle bacilli in the sputums with equal efficiency. There was, however, a decided difference in the ease of performance, speed and simplicity of the various individual methods. Sixteen different positive sputum smears consecutively stained and restained (after destaining) by nine different methods (Ziehl-Neelsen, Andrejew, Herman, Konrich, Gasis, Fontes, Burke and Dunning, Ehrlich, and Spengler) emphasized further the importance of technic in the staining of tubercle bacilli. Much's (Gram) method alone proved unsatisfactory with sputum on account of the difficulty of differentiating tubercle bacilli from other small cocci and dye precipitates, since in the majority of cases only the granules stain. Double bacillary staining (carbolfuchsin—Gram Much) of sputum and pus did not increase the efficiency of the simple single bacillary (carbolfuchsin) stain but increases the cumbersomeness of the technic. For practical purposes the simpler methods have the advantages of speed and simplicity. The finding of acid-fast bacilli (with the usual clinical precautions) practically certifies a diagnosis of tuberculosis by the simple methods, while the presence of Gram (Much) granules alone, requires corroborative positive animal inoculation findings to certify the diagnosis. It is for this reason that the Gram (Much) granules and combined methods are mainly of academic interest. It does not seem that the Gram granules differ from the granules seen in appropriately stained carbolfuchsin preparations since their numbers, morphology and location appear to be identical. Preparations stained first by the Gram (Much) method followed by the modified Ziehl-Neelsen carbolfuchsin-acid alcohol technic reveal only blue granules in a red bacillus. Red and blue granules in a lighter red bacillus were never noted. For practical purposes the simple steaming carbolfuchsin (Ziehl-Neelsen) method for staining tubercle bacilli, or one of its modifications, is recommended to the clinical pathologist. The choice of colors remains with the individual operator since any number of dye combinations can be used for staining and counterstaining tubercle bacilli. A negative finding by the simple method requires for further elaboration animal (guinea pig) inoculation, because as many as a million bacilli (about 0.000.1 mg.) per cubic centimeter material is hardly discernible microscopically while from 10 to 100 bacilli from a culture freshly isolated from sputum suffice to infect a guinea pig. In view of the danger of technical errors and in order to raise the present efficiency of the technic, especially with otherwise negative findings or when only single specimens are available, it is recommended that each specimen tested for tubercle bacilli by staining methods be controlled by

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the coincident staining of a like preparation to which a standard suspension of tubercle bacilli, which can easily be kept available in the laboratory at all times, has been added.

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THE REACTIONS OF TYPHOID VACCINATION*

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CLINICAL pathologists, in addition to other qualifications, are expected to be practical immunologists. At least, they are called upon to make and give various prophylactic and therapeutic vaccines and to make skin tests for susceptibility to various foreign substances and parasites. In many instances, it is easier to go through the motions than to explain the chemical and physical mechanism which determines the result. The practical results for the individual and for civilization also often speak for themselves, even in the absence of a complete analysis. But we lose our professional and scientific attitude and become mere technicians unless we try, no matter how unsuccessfully, to understand what we are doing.

The immunologic procedures for which we have the most convincing explanations are those concerned with antitoxic immunity as seen in the Schick and Dick tests and in the corresponding measures for active and passive immunization. Even here, however, the question of bacterial immunity is either ignored or disregarded, as the toxin-antitoxin factor is a decisive one in diphtheria and largely so in scarlet fever. The factor of bacterial immunity is still present, however, as is illustrated by healthy carriers of pathogenic diphtheria bacilli who have a positive Schick test. In other words, they have no antitoxic immunity but apparently plenty of antibacterial immunity. This factor is also illustrated by the septic complications of scarlet fever patients who have been cured of the toxic symptoms by active or passive antitoxic immunity. In the Schick and Dick tests we also have pseudo-reactions which increase with age and which have not been correlated with any immune processes. The early reactions in vaccination against smallpox are believed to be of significance in detecting immune individuals and to be parasitic in nature, but here we are dealing with a distinctive factor, namely, the living organism.

The recent advances in our knowledge of the problems of antitoxic immunity only emphasize our ignorance on questions of bacterial immunity, as is seen in typhoid vaccination. This procedure has prevented much sickness and death and made the world a safer and better place to live in, but many theoretical and practical questions remain.

At the Army Medical School during the last fifteen years, we have made a very large amount of typhoid vaccine, and naturally have seen and heard a good deal about reactions. We have heard about reactions following

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broken-off needle points, of reactions following intramuscular, intravenous and intracutaneous injections, and of reactions following doses varying from 1.0 c.c. upward. Of course, these are incidentals. The ordinary reactions in the average healthy person, even if not understood chemically, are well enough known clinically so that little need be said of them. Among the millions of persons injected with the vaccine from the School, a few unusual reactions have been reported. Even a few fatalities have been coincidental with, or more or less directly connected with, an injection of vaccine. Hence, we have probably had an unusual opportunity of trying to answer questions. In presenting some considerations on this subject, it is not with the hope of directly answering the many questions raised but with a view to restating problems which further work will eventually solve.

In the first place, can we tell who needs to be vaccinated or revaccinated? Undoubtedly, some persons are immune as a result of a previous attack of the disease or a previous vaccination, or possibly by reason of hereditary make-up, or on account of age. Various tests for specific immunity have been proposed, but these tests are not accepted today as sufficiently reliable for the selection of susceptibles. The skin tests are complicated by nonspecific factors and no great confidence can be placed in them. Even if purely specific we cannot interpret them in terms of real phagocytic or humoral immunity. An individual may have an hereditary horse asthma without being a good horseman, and in the same way a person may give reaction to substances in the typhoid bacillus without being able to master the bacillus in his tissues. There are antibodies in the serum, such as agglutinins, precipitins, and complement-fixing bodies, which we can measure, but we cannot say that their presence indicates protection, either clinically or experimentally.

If nonspecific and little understood specific factors complicate the question of bacterial immunity, the question of antitoxic immunity is just as difficult. No true toxins in appreciable quantities have been isolated and no potent antitoxin is available. To say "endotoxin" is to admit the whole difficulty. Gross variations in response to the toxic elements are easily demonstrated on different individuals, but the significance of these reactions in terms of immunity is not yet realized. The only answer that can be given to the questions, therefore, is one based on the clinical observation. A previous attack of the disease usually confers a high immunity for life and such persons need not be vaccinated. Of course, the actual nature of the previous infection should be authenticated, if possible. Well-vaccinated individuals are also protected for an indefinite time. In the army we now give two courses of three injections each with an interval of three years. At present, only clinical observation can tell us the limits of this acquired immunity. Some reliable test of immunity is much needed.

The second question which often arises is this: Does a sharp reaction indicate that the individual needs the vaccination, or the opposite? If we used the analogy of the Schick and Dick tests, a marked local and general reaction would be interpreted as an evidence of lack of immunity and the need of vaccination. But if we look at the reaction as antibacterial or aller-

gic, we would conclude that the patient is highly protected and does not need the vaccination. If we admit a third possibility, as we must, namely, one of nonspecific reaction, we reach the conclusion that these three factors are so intermingled that a satisfactory answer to this question cannot as yet be given.

There is a related question, namely: Is it possible to protect with a nontoxic vaccine? As far as can be told at present, the answer is in the negative. The so-called endotoxin seems in some way necessary for the immunization and has not been separated from the bacterial proteins. It is difficult to believe that a nontoxic vaccine can produce an immunity comparable with that which is acquired during the disease, only at the expense of weeks of severe sickness. We have worked with various fractions in order to try to separate the bacterial and toxic factors, but our purest bacterial fractions seem to have a toxic element, and our purest toxic fractions seem to have a bacterial element. Apparently these questions will remain unanswered until the physiologic chemist or physicist with an x-ray-like technic can see through the complexities of the structure of the bacillus and separate the fractions in usable forms.

Leaving these more fundamental questions, Major A. P. Hitchens and I have recently asked ourselves the more modest and practical question: Is it possible to foretell a severe reaction? In general, severe reactions are of two kinds, one which comes in a few hours, and the other which reaches its maximum effect in a few days. An example of the first is as follows:

"A man received his first injection November 28, 1923, without marked reaction. He had previously received two injections, one in 1915 and one in 1918. The reactions were always severe but not as bad as the present one. On December 5 he received a second dose at 10 A.M. One hour later, he had a chill. Two hours later he was very ill, cyanotic, pulse 100, weak and rapid, respiration 30, skin cold, and having a hard chill. He was put to bed and given 1.0 c.c. of adrenalin hypodermically. At 12 o'clock he vomited about two ounces of fresh blood. He vomited again at 12:30, and at 1 o'clock about one ounce of blood. Patient's color began to improve shortly after administration of adrenalin, and all alarming symptoms disappeared. At 2 o'clock his temperature was 104; from then on the patient recovered. The physicians who took care of the case thought it was doubtful if he would have recovered without prompt treatment."

This case, of course, cannot be considered as one of frank anaphylaxis, but we have had two other cases of similar nature, following the second dose, in which an allergic element must apparently be considered. Such cases should, of course, be differentiated from those in which the vaccine happens to enter a vein. The symptoms may be somewhat the same. True anaphylaxis has been reported with typhoid extracts in the guinea pig, but we have never been able to reproduce it with whole vaccine. We have not yet had or made an opportunity to try the passive transfer of anaphylaxis from a susceptible person to a nonsusceptible or to an animal. These reactions can at least be called anaphylactic-like, or anaphylactoid. They are extremely rare but the practical point is that adrenalin should be a part of the equipment for vaccination.

The second type is more toxic in nature, sometimes with symptoms of enteritis, which come on in twenty-four to forty-eight hours.

It may be said that in these reactions the vaccine is not at fault per se. Usually 20 to 200 men are vaccinated at the same time, from the same lot of vaccine, and only the occasional susceptible individual is picked out. As in the administration of arsphenamine or other drugs and serums, the difference in effect depends on the individual. This is reassuring to the vaccine maker, but does not help the vaccinator.

In pursuing the plan of attempting to pick out the bad reactions, we made intracutaneous injections in the arm of ordinary vaccine diluted at least to 100,000 organisms for the skin dose, and read the immediate reactions as in sensitization tests and the late reactions after twenty-four to forty-eight hours as possible indicators of the two types of reactions. We then attempted to correlate these reactions with various factors.

The results are given in Table I:

TABLE I

IMMEDIATE AND LATE SKIN REACTIONS IN RELATION TO REACTIONS FOLLOWING TYPHOID VACCINATION

(Suspension of Vaccine, 100,000 Bacilli in 0.1 c.c.)

	NO.	IMMEDIATE REACTION	24-HOUR REACTION
Previous typhoid fever	8	2.2	1.7
No typhoid or vaccination	21	1.	1.9
Previous vaccination	59	1.2	1.2
Other protein sensitizations	6	1.5	1.5
Before vaccination	28	1.2	0.8
3 months after	28	1.8	1.8

Figures indicate average size of reaction based on four plus system of reading.

The immediate skin reaction was marked in those who had had typhoid fever. Recent vaccination also increases the immediate reaction. Other sensitiveness also tends to increase the immediate reaction. These results give some indication of allergy, but they are not marked or entirely consistent. The late or toxic reactions are also suggestive, as those persons with no history of typhoid or vaccination give low immediate skin reactions and high late reactions. This conclusion does not hold true generally, however, as those recently vaccinated also give good late reactions. The most that can be said is that the results are suggestive of two factors, one bacterial and the other toxic.

The most definite correlation was between the immediate skin reaction and the general vaccination reaction, which ran fairly parallel. No connection could be seen between the late skin reaction and the vaccination reaction:

	IMMEDIATE SKIN REACTIONS	VACCINATION REACTION
(−) or single plus	13	31%
double plus or triple plus	21	65%

In other words, the allergic factor seems to be the most important in reactions. This conclusion fits in with clinical experience, which tells us that severe reactions are more apt to occur in those who have had typhoid fever or have been repeatedly vaccinated. It must be noted, however, that nonspecific reactions increase in severity with age, and the subject remains complex.

It has been thought that the paratyphoid fractions are more toxic than the typhoid fraction, and the Navy at present uses a monovalent typhoid vaccine of 1,000 million per c.c. We have tried the three fractions by skin reactions and by vaccination in separate arms and have been unable to find any essential differences.

In view of the apparently toxic and antitoxic element in some individuals, we have attempted to neutralize the toxic factor by mixing vaccine and serum from apparently antitoxic individuals and trying skin tests on susceptibles. Six tests with proper controls were made in which four showed a definite neutralization of toxin with serum from a nonreactor. The nature of this neutralization is difficult to explain as antiendotoxins are not generally accepted. The subject is complicated by sensitiveness of the skin of some individuals to the injection of serum or even of salt solution.

One definite conclusion is the obvious one that the strength of the vaccine materially affects both early and late reactions. Soon after the war, we revised our plant and technic and put the vaccine on the theoretical strength of 2,500 m. per c.c., assuming that the Counting Chamber technic gave the same results as the Wright method. We immediately found that the number of reactions increased. We have since found that the Counting Chamber technic gives a stronger vaccine, because the count is lower and the dilution less. In the Wright technic many red cells are lost and the dilution is greater than it should be. We have now reduced our vaccine to 1,000 m. per c.c.; 500 m. typhoid, 250 m. *A*, and 250 m. *B*, which is equivalent to a count of about 2,000 by the Wright technic, and we believe more nearly accurate. Practically all complaints have ceased since this reduction.

In general, it can be said that there are at least three factors in the reactions: one specific and bacterial, a second specific and toxic, and a third which is nonspecific. While one or the other element may be shown to predominate in the picture, they cannot be sharply separated. The practical conclusion is that the clinical pathologist has the arduous occupation of being both a clinician and a pathologist. We must individualize our subjects for vaccination and be prepared for various reactions. In case of doubt, the usual procedure is to cut the dose and increase the number of injections. We have analogy in support of this procedure but no actual figures on protection. A skin test may help in detecting severe reactors.

In clearing up some of these questions, the following lines of work seem to be the most promising:

1. The separation of distinct fractions of the bacillus by competent chemists.

2. The skin test of these fractions with proper controls on typhoid fever patients and convalescents and in normals of different ages and on vaccinated individuals.

3. Attempts to transfer and neutralize skin reactions by using serum from reactors and nonreactors.

4. A more critical clinical study of ordinary reactions.

SUMMARY

1. Recent advances in dealing with antitoxic immunity as seen in diphtheria and scarlet fever have not been duplicated in regard to bacterial immunity as seen in typhoid fever.

(a) It is not possible at present to detect susceptibles and immunes to typhoid fever by skin or other tests.

(b) The significance of reactions to vaccination in terms of immunity is not yet clear.

(c) The question of toxic and nontoxic vaccines is not yet satisfactorily answered.

2. Severe vaccination reactions are of two kinds, immediate or anaphylactic-like, and late or toxic.

3. Immediate and late skin reactions with vaccine give some suggestions of two factors in reactions, (a) bacterial or allergic, (b) toxic.

4. There is also a marked nonspecific element.

5. These factors are so mixed together that an analysis of reactions in terms of immunity is not possible at present.

6. Severe reactors may in some instances be picked out by immediate skin reactions.

DISCUSSION

Dr. Frank M. Huntoon.—Every now and then some one comes along with common sense, and it rather startles us. There are many things upon which one could disagree with Dr. Nichols, on his theoretical ground, and on his question whether the toxic element is necessary for immunization. Neither of us has any proof. I would not give up hope of producing such a vaccine. It seems to me that the old problem of the nature of the immunity produced here by the natural disease or by immunization lies in the question that Dr. Kolmer brought up: Is it a strictly humoral immunity? This again leads to the question of the method of infection in typhoid fever. Is typhoid primarily a disease of the intestines or does it get to the intestines through the blood stream? Now there is another question which may be brought up: Is it necessary to have antibody in the blood in order that the patient may be immune from the actual typhoid? I do not think so. It is a fact that you can inject an animal with typhoid serum and measure the time it takes for antibodies to appear in the blood. If you allow that animal to remain untreated until the antibodies have disappeared, and then if you reinject it and test it every day to see when the antibodies reappear, you can probably find them in twenty-four hours. The paper by Dr. Nichols points the way to some very useful and needed work, work that does not have to be done in highly organized, complete laboratories, but anywhere.

Dr. A. S. Brumbaugh.—In 1918 at the Army Medical School, one of the medical officers originated a typhoid vaccine made up in oil that was supposed to have the advantage that the whole immunization could be administered at one time and which was not supposed to cause any reaction. I would like for Dr. Nichols to tell us what has been done about that.

Dr. Robert A. Kilduffe.—I do not wish to discuss this paper; as usual, I am in search of information.

Dr. Nichols has called our attention to the fact that it is a very difficult thing to measure in any way the degree of immunity to typhoid fever, and it is well known that the agglutinin-titer is not a measure of immunity.

Sometime ago Dr. O'Hara, of Boston, described the study of a small series of typhoid fever cases by frequent quantitative agglutination tests and suggested, as a result of his

observations, that when the agglutinin-titer began to drop in the face of unchanged or unfavorable clinical findings, that the dropping titer was a possible indication of an unfavorable prognosis.

I was fortunate enough to be able to try this out and, though my series was very limited, this suggestion was apparently borne out: the cases in which the titer showed a sudden or persistent drop, died.

I have never seen any further report or note upon this phase of the agglutination test in typhoid fever. Knowing the immense field covered by the records of the army in this branch of work, I would like to ask Dr. Nichols if any work has been done in the army along this line or if they have any information as to the availability of this procedure as a possible prognostic criterion.

Dr. Robert A. Keilty.—We are continuing to give typhoid vaccine without thinking of fatality. There were possibly a million doses given, with one fatality, and I do not know whether it is in the records or not. In four autopsies there was nothing at all to account for death.

Dr. John A. Kolmer.—One subject of considerable practical importance in relation to Dr. Nichols' paper is the matter of developing a satisfactory test for natural or acquired immunity in typhoid fever. I agree with Dr. Nichols that the skin test has not fulfilled expectations and hopes in this direction; I also doubt the value of the agglutinin reaction for this purpose, but, inasmuch as a goodly part of immunity to the typhoid and paratyphoid bacilli would appear to be humoral in nature, I am hopeful that investigations will ultimately evolve a bactericidal test in vitro applicable to the whole blood as a satisfactory measure of immunity to serve as a practical guide to vaccination and revaccination in this disease.

Dr. H. J. Nichols (closing).—Dr. Kolmer has spoken of the typhoidin test; it is certainly to be hoped and expected that we will soon have some kind of skin test which will indicate the necessity or lack of it for vaccination. Experimentally, protection tests are the most convincing. Determination of antibodies is not so conclusive. The guinea pig has a natural infection with a Para *B*-like organism, B. aertrycke, and it is possible to carry out actual protective experiments under different conditions with this organism. We have done some of this work and reported it in the Jour. Exp. Med., 1923, xxxviii, 283. Lipovaccine has been referred to. This was given up on account of difficulty of making a sterile product and a poor showing experimentally.

We have had several fatalities, such as have been mentioned; in one we found a very high degree of calcification of the coronary artery. One occurred among a number of men who had food poisoning at the time of vaccination. All our evidence in these cases points to some special weakness in the individual rather than defect in the vaccine and argues for individualizing our prophylactic as well as our therapeutic vaccines.

I feel that we are actually just starting upon a real understanding of this general subject. As Dr. Huntoon says, it does not require any elaborate technic or equipment to progress. We will get somewhere if we simply observe with open eyes. Of course, all that is learned in the finer analysis of reactions like the tuberculin test and other skin tests will help to give the final answer.

BLOOD COUNTS IN MISSISSIPPI*

By Leon S. Lippincott, M.D., Vicksburg, Miss.

IN THE last few years several observers have called attention to the fact that there is no standard normal total leucocyte count or differential leucocyte count that holds good for all localities. It was in the belief that an investigation of differences should be carried further and with the idea of establishing standards for our work in Mississippi that the study covered by this paper was attempted.

Most textbooks give the total leucocyte range for normal individuals as from 5,000 to 10,000. The normal differential count is given as varying as follows:

Small mononuclears	20 to 25%
Large mononuclears (including transitionals)	2 to 11%
Polymorph. neutrophiles	60 to 75%
Polymorph. eosinophiles	1 to 5%
Polymorph. basophiles	0.5%

The counts here considered, 3,045 in number, were made on hospital and clinic patients, many of them as a part of the routine laboratory examinations. The records were taken as they came, with no attempt to pick cases. The differential counts were made from Wright's stain preparations. Counts with eosinophile percentages above five were not included as such counts in our experience generally indicate animal parasites. Counts from white and colored patients were listed separately to determine any possible differences due to race. Children's counts were not included.

Total leucocyte counts within the so-called normal range of 5,000 to 10,000 were first considered, with the following average findings:

Averages of 1500 Counts on White Patients Showing Leucocytes 5,000 to 10,000	
Total leucocyte count	7,638
Small mononuclears	30.60%
Large mononuclears	6.50%
Polymorph. neutrophiles	61.85%
Polymorph. eosinophiles	0.95%
Polymorph. basophiles	0.10%

Averages of 300 Counts on Colored Patients Showing Leucocytes 5,000 to 10,000	
Total leucocyte count	7,395
Small mononuclears	33.55%
Large mononuclears	4.95%
Polymorph. neutrophiles	60.50%
Polymorph. eosinophiles	0.95%
Polymorph. basophiles	0.05%

It will be noted that the small mononuclears are considerably above the usual maximum normal standard and the polymorphonuclear neutrophiles

*Read before the Fourth Annual Convention of the American Society of Clinical Pathologists at Philadelphia, May 20 to 23, 1925.

near the usual normal lower limit. The average eosinophile percentage is less than one, although actually we rarely are able to demonstrate intestinal parasites unless the eosinophile percentage is five or more. The basophiles are much lower than the usual standard normal.

As the above counts are from hospital and clinic patients, it is possible that there may have been inflammatory conditions present in some instances, which would raise the neutrophile percentage; and malaria or typhoid in others, which would lower it. It is felt that these two conditions would possibly offset each other.

From observation of clinical conditions and findings I am convinced that in a great majority of cases a total leucocyte count above 8,000 in Mississippi means a leucocytosis and a neutrophile percentage above 65 means an increase over normal. In making this statement, I do not ignore the fact that there are normal variations.

There appears to be no marked difference between the counts of white and colored patients. The mononuclears are slightly lower in whites and the polymorphonuclear neutrophiles slightly higher. Basophiles were scarce in colored patients.

At least one observer has noted that persons who have lived six weeks or longer in high altitudes show a low leucocyte count with increased mononuclears and low polymorphonuclear neutrophiles. Altitude could not be considered as a factor in the counts recorded here as Mississippi is generally low.

TOTAL LEUCOCYTE COUNTS BELOW 5,000

When counts below 5,000 are considered, the average total leucocyte counts and differential counts are found to be as follows:

AVERAGE OF 100 COUNTS ON WHITE PATIENTS SHOWING LEUCOCYTES BELOW 5,000

Total leucocyte count		4,182
Small mononuclears	35.1%	
Large mononuclears	11.5%	
Polymorph. neutrophiles	52.5%	
Polymorph. eosinophiles	0.8%	
Polymorph. basophiles	0.1%	

AVERAGE OF 5 COUNTS ON COLORED PATIENTS SHOWING LEUCOCYTES BELOW 5,000

Total leucocyte count		4,450
Small mononuclears	34.0%	
Large mononuclears	6.2%	
Polymorph. neutrophiles	58.4%	
Polymorph. eosinophiles	1.4%	
Polymorph. basophiles	0.0%	

In these low counts the mononuclears are increased at the expense of the neutrophiles. Basophiles were again low.

TOTAL LEUCOCYTE COUNTS ABOVE 10,000

Counts above 10,000 are considered in three groups: those from 10,000 to 15,000; those from 15,000 to 20,000; and those above 20,000, the counts of white and colored patients being given separately, as follows:

AVERAGE OF 500 COUNTS ON WHITE PATIENTS SHOWING LEUCOCYTES 10,000 TO 15,000

Total leucocyte count	11,604
Small mononuclears	19.8%
Large mononuclears	5.0%
Polymorph. neutrophiles	74.6%
Polymorph. eosinophiles	0.5%
Polymorph. basophiles	0.1%

AVERAGE OF 100 COUNTS ON COLORED PATIENTS SHOWING LEUCOCYTES 10,000 TO 15,000

Total leucocyte count	12,448
Small mononuclears	21.4%
Large mononuclears	5.4%
Polymorph. neutrophiles	72.7%
Polymorph. eosinophiles	0.5%
Polymorph. basophiles	0.0%

AVERAGE OF 300 COUNTS ON WHITE PATIENTS SHOWING LEUCOCYTES 15,000 TO 20,000

Total leucocyte count	16,905
Small mononuclears	15.3%
Large mononuclears	6.2%
Polymorph. neutrophiles	78.0%
Polymorph. eosinophiles	0.4%
Polymorph. basophiles	0.1%

AVERAGE OF 20 COUNTS ON COLORED PATIENTS SHOWING LEUCOCYTES 15,000 TO 20,000

Total leucocyte count	17,840
Small mononuclears	16.1%
Large mononuclears	3.9%
Polymorph. neutrophiles	79.5%
Polymorph. eosinophiles	0.3%
Polymorph. basophiles	0.2%

AVERAGE OF 200 COUNTS ON WHITE PATIENTS SHOWING LEUCOCYTES ABOVE 20,000

Total leucocyte count	26,640
Small mononuclears	10.7%
Large mononuclears	6.1%
Polymorph. neutrophiles	82.7%
Polymorph. eosinophiles	0.4%
Polymorph. basophiles	0.1%

AVERAGE OF 20 COUNTS ON COLORED PATIENTS SHOWING LEUCOCYTES ABOVE 20,000

Total leucocyte count	25,404
Small mononuclears	15.1%
Large mononuclears	5.2%
Polymorph. neutrophiles	79.2%
Polymorph. eosinophiles	0.5%
Polymorph. basophiles	0.0%

There is nothing especially remarkable in any of these higher counts. The polymorphonuclear neutrophiles increase at the expense of the mononuclears as the total count increases, although the average neutrophile count for all cases is not as high as most observers in other localities find with similar leucocyte counts. This is in accord with what we consider a normal neutrophile percentage. The eosinophile and basophile counts are low, the latter especially so in the negroes studied.

CONCLUSIONS

Studies in Mississippi indicate:

1. The normal average total leucocyte count in this locality is between 7,000 and 8,000.

2. The small mononuclear percentage is normally higher than the usually accepted maximum standard.

3. The polymorphonuclear neutrophiles percentage is near the usually accepted normal minimum standard.

4. The normal eosinophile count is less than 1 per cent.

5. The normal basophile count is less than the usual standard of 0.5 per cent, and especially low in negroes.

6. In total counts below 5,000, the mononuclear percentages are increased at the expense of the neutrophiles.

7. In total counts above 10,000, the polymorphonuclear neutrophiles are in general lower than would be expected from textbook descriptions.

8. There is no marked difference in total leucocyte counts or differential leucocyte counts in whites and negroes, except that basophile percentages are lower in negroes.

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DISCUSSION

Dr. James B. Bullitt.—I have been very much interested in Dr. Lippincott's paper. I lived in Mississippi for ten years and made a great many blood counts. My experience while there was very closely in accord with his. A very large percentage of the people had, or recently had had malaria. At least fifteen to twenty per cent of the persons whom I examined had rather a high eosinophile count. In a large number of the cases it was the result of hookworm infection. In the eosinophile count there were from three or four up to fifteen per cent, usually in the neighborhood of four or five per cent. My mononuclear counts were very much the same as those of Dr. Lippincott. This condition was, in my judgment, due to active or latent malaria.

Dr. Ralph G. Stillman.—While I have no figures, I am inclined to think that this lowered polymorphonuclear percentage is not characteristic of the South. My experience in New York has been that in apparently normal individuals the poly percentage is lower than that given in most of the textbooks and approximates the figures given in Dr. Lippincott's paper.

Dr. W. Parker Stowe.—Several years ago in studying the relation of neutrophilic polynucleosis to total leucocytosis I ran over three hundred normals to establish a base line. It is perhaps interesting that I arrived at the same figures for the normal white count in Rochester, New York that Dr. Lippincott has mentioned for Mississippi, i. e., 8,500 total white count with 65 per cent neutrophiles.

Dr. Leon S. Lippincott (closing).—If the figures in the textbooks are wrong they should be changed. If they are not wrong then the difference in the different parts of the country should be stated. I want to defend Mississippi somewhat on one point. If you go to the statistics as reported by the State Board of Health, you will find that Mississippi is overrun by malaria. This is due to the fact that malaria is often reported with blood examination. Many of the patients make their own diagnoses. Comparatively few cases are actually malaria. There is not nearly as much malaria in Mississippi as is supposed. In regard to the eosinophile count, I expected to find higher eosinophile counts but did not find them except in those cases in which we were able to demonstrate animal parasites. When we find 5 per cent or more eosinophiles we are usually able to find hookworm or other parasites. We see hardly any hookworm in my district.

CHEMICAL INVESTIGATIONS ON NEOARSPHENAMINE*

I. Methods of Analysis

By Louis Freedman, Ph.D., Brooklyn, N. Y.

ALTHOUGH neoarsphenamine has now been in extensive clinical use for over ten years and its therapeutic importance has long been established, the published work on its analysis and chemical composition is both limited and inconclusive. Neoarsphenamine was first described in the patent literature[1] as a condensation product of salvarsan with sodium formaldehyde sulphoxylate, while the circulars accompanying the original preparation, as manufactured by the Farbwerke Hoechst in Germany, described it as the monomethylene sulphinate of salvarsan. The lowered arsenic content of 19 per cent from the theoretical of 32 per cent was accounted for by the presence of inert salts. Commercial preparations of neoarsphenamine are generally described as "a compound formed from arsphenamine by the action of formaldehyde sulphoxylate." The above description has been generally adopted by all manufacturers of the drug, and today any product which passes the required toxicity tests and conforms to an arsenic content of at least 19 per cent, regardless of modes or conditions of manufacture, is accepted as neoarsphenamine.

Bertheim in 1913[2] called attention to the possibility of the combination of one or two mols of formaldehyde sulphoxylate with salvarsan resulting in either a mono- or disubstituted product, depending upon the conditions of manufacture.

In 1921, Raiziss and Falkov[3] described a method for the examination of neoarsphenamine from a chemical standpoint and concluded that neoarsphenamine contained both the mono- and disubstituted derivatives. In 1921 and 1922, Macallum[4, 5] published reports on the chemical analysis of domestic and foreign neoarsphenamines and found that there was considerable difference between the American and French products, and that the latter were made up almost entirely of disubstituted derivatives. Voegtlin and Miller[6] in 1922 showed by trypanocidal and toxicologic tests that very wide variations existed for different preparations of neoarsphenamine and emphasized the lack of uniformity in the products. In 1922, Dale and White,[7] in their report to the British Medical Research Council on the biological efficacy of various preparations of the salvarsans, stated that preparations of the neosalvarsan or "914" type fell into two distinct classes. In the first class were those which resembled the German product more or less closely in certain physical characteristics and the rapidity with which they underwent decomposition on exposure to air. The second group consisted of those products which differed from the German product in being more easily soluble and showing evidence of greater stability in solution. The compounds of the second group were described by Dale and

*From the H. A. Metz Laboratories, Inc., Brooklyn, N. Y.
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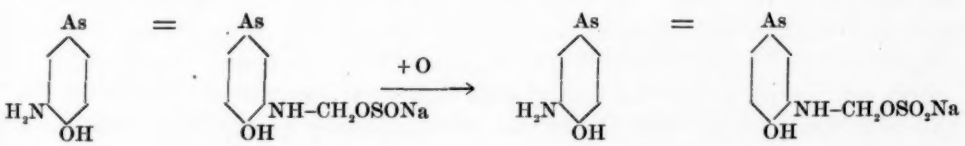
White as being of lower toxicity but also inferior in therapeutic activity as compared with the original product of German manufacture.

It would seem, therefore, that these wide variations in the composition of various brands of neoarsphenamine were in part due to the fact that the manufacturers supplemented the specifications of the original method of manufacture, primarily to obtain products of low toxicity, but without much regard to the identity of the resulting products, provided they conformed to the existent requirements. A comparison of the method of preparation described in the original patent specifications (l.c.) with those of Krumweide[8] and Oechslin[9] show how widely they may differ.

It is quite obvious, therefore, that the name neoarsphenamine is used to cover a number of products which are prepared from the same chemicals, but which may differ radically in some of their physical properties and in their chemical composition. These differences are also reflected in wide variations in toxicity and trypanocidal efficiency. It is therefore of great importance for a method of analysis to be developed which will serve to distinguish the different types of neoarsphenamines.

Those authors who published analytical data on neoarsphenamine with the purpose of determining the chemical constitution of the drug, include A. D. Macallum (l.c.), Raiziss and Falkov (l.c.), de Mytennaere[10] and Elvove.[11, 12] Macallum, in his first paper, "Examination of Neoarsphenamines" (l.c.), devised a method for the determination of the relative amounts of the various constituents by a portionwise oxidation of the drug by means of iodine. He found that in the presence of strong mineral acids neoarsphenamine required 12 mols of iodine for complete oxidation, indicating that the formaldehyde sulphoxylate side chain was broken off and completely oxidized. In his second paper, "The Constitution of the French Drugs," Macallum extended his method of analysis by carrying out the procedure successively in acid and alkaline solutions. He claimed by this method to be able to differentiate between the combined formaldehyde sulphoxylate and bisulphite groups and also to determine the varying amounts of these uncombined formaldehyde sulphur compounds. Macallum also stated that the first step in the oxidation of neoarsphenamine was the oxidation of the N-formaldehyde sulphoxylate group to the N-formaldehyde bisulphite group according to the equation,



and he included the two compounds represented by the above formulae, under the name of neoarsphenamine.

Raiziss and Falkov's (l.c.) method for the calculation of the various constituents of commercial neoarsphenamines was based on a partition of sulphur in the drug, and is to a certain extent similar to Macallum's method. The total sulphur was first determined by both the Carius method and oxidation by digesting with iodine for six hours. The difference between the two found

values was accounted for as nuclear sulphur, although no evidence was given that nuclear sulphur was not oxidized by digestion with iodine. The percentages of nuclear sulphur were found to be so small that they may actually have come within the limits of experimental error.

The sulphur of the uncombined formaldehyde sulphoxylate, together with any inorganic sulphates, was determined by oxidizing the filtrate obtained from the precipitation of the free neosulphinic acid by means of hydrochloric acid. The sulphur of the combined sulphoxylate was represented as the difference between the total sulphur (exclusive of nuclear sulphur) and the sulphur of the filtrate. Arsenic was determined gravimetrically. From the results of their analyses, Raiziss and Falkov concluded that neoarsphenamines were made up of mixtures of mono- and disubstituted derivatives and tabulated the various percentages of these constituents, together with the amounts of combined and uncombined formaldehyde sulphoxylates, although they failed to state how they arrived at their conclusions. The authors also failed to observe, as de Mytennaere (l.c.) pointed out in a polemic on their paper, that a certain percentage of the combined sulphur may have been in the form of the oxidized sulpharsphenamine.

In the results recorded by Raiziss and Falkov the sum of the sulphur contents of the various constituents fails to check with the total sulphur found, and many of the calculations do not check arithmetically. For example, in Table V, page 217, for sample D1, the total combined sulphoxylate was found to be 18.6 mg., but when calculated from the figures given for mono- and disubstituted neoarsphenamines, it is equivalent to only 15.9 mg. The total organic arsenical component of D1 was given as 63.8 mg., but from the arsenic content of the sample, which is equivalent to 48 mg. arsphenamine, plus the 18.6 mg. combined sulphoxylate found by analysis, the figure should total 66.6 mg.

Raiziss and Falkov have also stated that the formaldehyde sulphoxylate attached to the amino group is not oxidized by iodine in the cold. This is contrary to the statement of Macallum (l.c.) and also contrary to our own experimental findings, which will be given in detail in the experimental part of this paper.

De Mytennaere (l.c.) studied the chemistry of various neoarsphenamines from the standpoint of the effect of the variation in chemical constitution on the toxicity of the drug. He determined the percentages of carbon, hydrogen, nitrogen, arsenic and sulphur in various commercial samples and also attempted to precipitate the various constituents by fractional precipitation in acetic acid solution, but his results were not very conclusive. He stated that the free neosulphinic acid could not be completely precipitated from compounds containing 10 per cent or more of sulphur; and that he found very little difference between neoarsphenamine and sulpharsenol (sulpharsphenamine).

Elvove (l.c.) described a method for the estimation of sulphur as sulphate in neoarsphenamine, for the purpose of determining, together with other analytical data, the distribution of sulphur in the drug. He (l.c.) also described a method for the determination of the total sulphur in neoarsphenamine and sulpharsphenamine which depends on the oxidation of the sample

by means of potassium permanganate and hydrochloric acid. This latter method is said to have the advantage over the Carius or peroxide-fusion method because of its simplicity of operation and because the procedure is similar to that used for the determination of arsenic, so that the latter may be determined in the same sample of the drug.*

The above articles include practically all the available data regarding the investigations of neoarsphenamine from a chemical standpoint. In the experimental part which follows, we have, by attempting to apply these methods, been able to show that certain of them are based on certain fallacies and consequently cannot give accurate results.

EXPERIMENTAL PART

In the study of a series of different commercial preparations of neoarsphenamines which were under investigation in this laboratory, we attempted to utilize the methods described by both Macallum, and Raiziss and Falkov, by combining the essential operations of both methods without changing them in principle. In this way the analyses were carried out with a minimum amount of manipulation and the use of a minimum amount of material.

Arsenic was determined according to Lehmann's method (l.c.), and the total sulphur by Elvove's (l.c.) method. The total oxygen requirement, which is the same as the total reducing power in Macallum's method, was obtained by titration of 0.2 gram of the sample dissolved in 30 c.c. air-free water, with 0.1 N iodine solution. One c.c. 0.1 N I is equivalent to 0.8 mg. oxygen. All precautions to avoid possibility of oxidation by the air were taken. The oxygen requirements of the filtrates, i.e., of the uncombined sodium formaldehyde sulphoxylate, were obtained, precisely as described by Macallum (l.c.) and Raiziss and Falkov (l.c.), by precipitating the free neosulphinic acid with dilute hydrochloric acid and titrating the filtrates with the standard iodine solution. The analyses for the sulphur partition of the samples, according to the method of Raiziss and Falkov were also made on portions of these same filtrates. The results obtained by the above combined methods of analyses, which are given in Table I, did not give satisfactory and concordant results as claimed by the authors of these methods. The inconsistencies in the above results, together with many striking observations made during the course of the analyses, prove the impracticability of the methods. We found that the amount of iodine required for complete oxidation of neoarsphenamines increased as the acidity of the solution increased; that different amounts of acid were required to precipitate completely the different free sulphinic acids from their respective sodium salts;† and that at least one brand of neoarsphenamine could not be completely precipitated except by the use of concentrated hydrochloric acid and allowing the solution to stand until the filtrates no longer became turbid. We have also found that the determination of sulphur in the filtrates has no quantitative value, because the neosulphinic acid is more or less unstable in acids strong enough to make a precipitation anywhere near com-

*A third paper by Elvove[13], "A Method for the Examination of Neoarsphenamine and Sulpharsphenamine," appeared after this article was written. This paper will be discussed by us in a subsequent publication.

†The free acid precipitated from aqueous solutions of neoarsphenamine will be referred to hereafter as neosulphinic acid.

plete, the formaldehyde group being split off the N-organic nucleus with the formation of arsphenamine hydrochloride.

As the methods of Macallum and Raiziss and Falkov are based directly on the assumptions that arseno groups can be titrated quantitatively by iodine in an acid medium and that the free sulphinic acid of neoarsphenamine is quantitatively precipitated by means of hydrochloric acid without decomposition, it is evident that these methods cannot give quantitative results.

Since some of the figures given in Table I are obviously incorrect, we did not use them as a basis for calculating the various constituents of the sample as detailed in the method of Raiziss and Falkov (l.c.). In this table the difference between the total oxygen requirements (Column 5) and that required by the trivalent arsenic (Column 6) is the oxygen required by the uncombined formaldehyde sulphoxylate (Column 7). This should check with the oxygen requirements of the uncombined formaldehyde sulphoxylate as calculated by titration of the filtrates with iodine (Column 9). In all of the samples, with the exception of Sample F, the oxygen obtained by calculation (Column 7) is greater than that obtained by actual titration, this difference (Column 10) varying considerably in each case.

Assuming that commercial neoarsphenamines, besides the actual arseno compound and sodium formaldehyde sulphoxylate, contain nothing which will be oxidized by iodine, the combined formaldehyde sulphoxylate groups attached to the amino groups therefore must react with iodine, being oxidized to the formaldehyde bisulphite groups. Thus a disubstituted neoarsphenamine should require more iodine than a monosubstituted product. This difference in oxygen requirements (Column 10), however, does not check with the calculated combined formaldehyde sulphoxylate for each sample of neoarsphenamine, even though it is assumed that only monosubstituted neoarsphenamine is present. It is therefore evident that some other factor or factors are involved in this method of analysis which tends to give such discordant results.

EFFECT OF ACIDITY ON TITRATIONS OF ARSENO GROUPS WITH IODINE

The variability of the results obtained by the previously described methods led us to investigate the effect of acidity on the titrations of the arsenicals with iodine, both with regard to the arseno groups alone and when combined with side chain groups, as in neoarsphenamine.

It has been definitely shown that the trivalent arsenic in arsphenamine is practically quantitatively oxidized to the pentavalent arsenic acid by means of iodine in neutral or very slightly acid solutions.[15, 16] In the presence of an excess amount of mineral acid, however, we have found that the oxygen requirement of the arseno group decreases, less iodine being required for complete oxidation to pentavalent arsenic, and that this decrease is proportional to the increase in acidity of the titrated solution.

Table II shows that, when the titration with iodine is carried out in a solution, the final acidity of which is approximately 1.0 per cent, only 97 per cent of the theoretic iodine is required. When the acidity is further increased to 3.24 per cent, 91 per cent of the theoretic iodine is required to completely

TABLE I

ANALYTICAL DATA OF COMMERCIAL NEOARSPHENAMINES OBTAINED BY COMBINATION OF METHODS OF MACALLUM AND RAIZISS AND FALKOV

SAMPLE OF NEOARSPHENAMINE*	% AS IN ORIG. SAMPLE	% S IN ORIG. SAMPLE	C.C. N/10 I PER 100 MG. OF SAMPLE	MG. OXYGEN REQUIRED PER 100 MG. NEOARSPHENAMINE			C.C. N/10 I REQ. BY FILTRATE PER 100 MG. NEO.	OX. REQ. BY FILTRATE CALC. EX. I TITRATION	MG. OX. DIFF. BETWEEN CALC. AND FOUND.	SULPHUR DISTRIBUTED IN FILTRATE			
				TOTAL OX. CALC. EX. I REQ.	OX. CALC. EX. AS	DIFFERENCE OX. REQ. BY FILTRATE				TOTAL S CALC. BY DIFF.	TOTAL S FOUND	S FOUND AS Na_2SO_4	S AS FREE Na F. S. OX., Na F. B. S., ETC.
A	19.26	11.57	17.63	14.11	8.21	5.90	5.73	4.58	1.32	7.46	8.46	2.06	6.40
B	20.15	11.35	17.08	14.46	8.60	5.86	5.50	4.40	1.46	7.06	8.18	3.23	4.95
C	20.06	7.23	16.78	13.44	8.78	4.66	4.98	3.98	0.68	2.96	5.04	0.87	4.17
D	18.85	10.55	18.03	14.42	8.04	6.38	5.17	4.14	2.24	6.54	6.85	2.33	4.52
E	19.22	9.04	16.33	13.06	8.20	4.86	4.08	3.27	1.59	4.84	6.61	1.88	4.73
F	19.68	11.07	11.48	9.18	8.39	0.79	1.25	1.00	−0.21	6.87	4.35	0.56	3.79
G	19.65	9.01	16.46	13.16	8.37	4.79	4.69	3.75	1.04	4.82	6.49	1.71	4.78

*These samples are representative of the different commercial brands of neoarsphenamine obtainable in the open market.

Abbreviations: Ox. = Oxygen; Req. = Requirements; Calc. = Calculated; ex. = from; Na F.S.Ox. = Sodium Formaldehyde sulphoxylate; Na F.B.S. = Sodium formaldehyde bisulphite.

TABLE II

EFFECT OF ACIDITY ON TITRATION OF ARSPHENAMINE WITH IODINE

AMOUNT OF SAMPLE IN GM.	AMOUNT OF HCl ADDED	FINAL ACIDITY OF SOLUTION AFTER ADDITION OF IODINE	N/10 I* REQUIRED PER 100 MG. ARSPHENAMINE	OXYGEN EQUIVALENT PER 100 MG.	DECREASE IN MG. OXYGEN REQUIRED	% DECREASE
0.2	0	0.5%†	16.35	13.08	–	–
0.2	0	0.5†	16.33	13.06	–	–
0.15	0	0.5†	16.40	13.12	–	–
0.2	23 c.c. 1.8%	0.87	16.16	12.92	0.21	1.5
0.2	30 " "	1.0	16.08	12.86	0.28	3.0
0.2	30 " "	1.0	16.00	12.80	0.34	3.0
0.1	10 " 18. %	3.24	15.00	12.00	1.14	9.0
0.1	20 " "	6.24	11.00	8.80	4.34	33.0
	ACETIC ACID					
0.1	20 c.c. 30%	10.0%	16.20	12.96	0.18	1.3
0.1	20 c.c. 100%	33.0	15.95	12.76	0.38	2.7

NOTE: Total volume of solution including added acid, but before addition of iodine, was 30 c.c. per 0.1 gm. arsphenamine. Excess iodine titrated back after 3 minutes, with standard thiosulphate solution using starch as indicator.

*The theoretical number of c.c. of N/10 I required for this sample containing 30.82 per cent As is 16.45, which is equivalent to 13.14 mg. oxygen.

†This acidity which is due to the HI formed by reduction of the iodine, together with that of the HCl of the arsphenamine hydrochloride, (which is so little as to be negligible) may be responsible for the slight decrease in oxygen requirement from the theoretical.

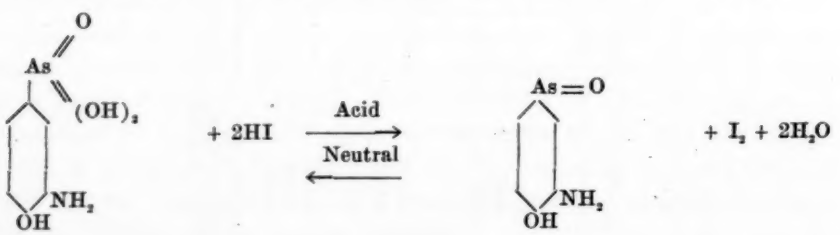
oxidize the arsenic; and in a solution containing approximately 6.0 per cent HCl, only 67 per cent of the theoretic iodine is required.

Acetic acid also has a tendency to decrease the amount of oxygen required by the trivalent arsenic, but to a much smaller degree than hydrochloric acid. Thus in a 33 per cent acetic acid solution, the amount of oxygen required by iodine titration was about 97 per cent of the theoretic, showing that acetic acid has very little effect.

These results indicate that the reaction between the arseno compound and iodine is a reversible one in the presence of mineral acids, similar to the reversible reaction taking place when arsenious acid is titrated with iodine in high hydrion concentration according to the equation:

$$H_3AsO_3 + I_2 + HO_2 \underset{\text{Acid}}{\overset{\text{Neutral}}{\rightleftharpoons}} H_3AsO_4 + 2HI$$ [17, 18]

The corresponding reaction of the arsenic acid may be written thus:



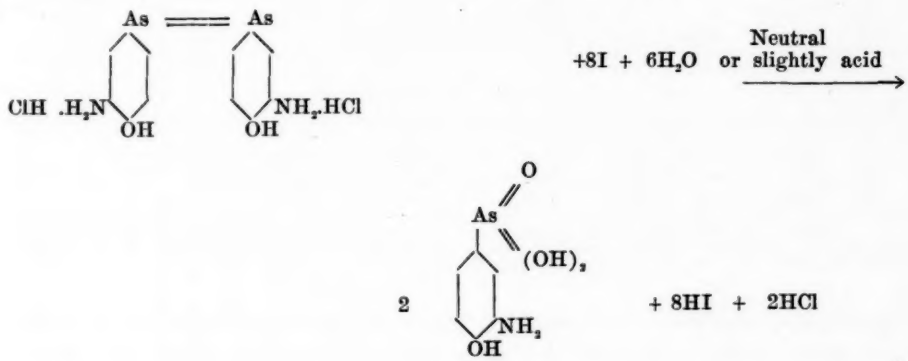
EFFECT OF ACIDITY ON TITRATIONS OF NEOARSPHENAMINE WITH IODINE

As neoarsphenamine is supposed to contain the same arseno grouping as arsphenamine, we would expect to get similar results on titration of solutions of neoarsphenamine with iodine as we get with solutions of arsphenamine.

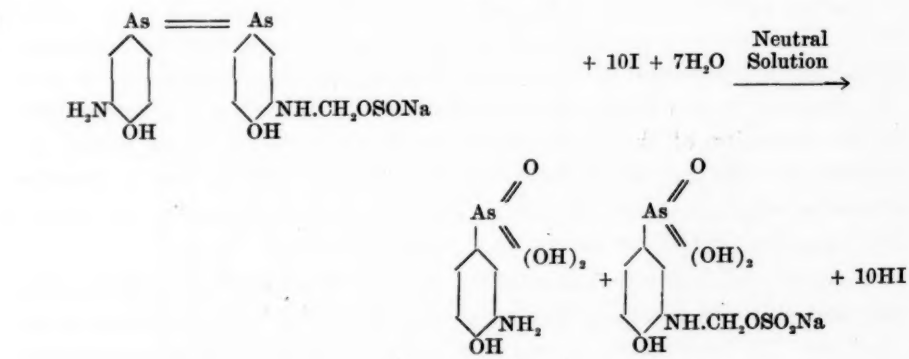
However, actual experimental results show the reverse to be true, i.e., slightly more iodine is required as the concentration of acid is increased. This is due, not to any different reaction taking place with regard to the trivalent arsenic groups, but to the fact that the combined N-formaldehyde-sulphoxylate group is progressively hydrolyzed as the concentration of hydrions increases and the liberated formaldehyde-sulphoxylate group is then completely oxidized by the iodine. The decrease in oxygen requirements caused by the reversible reaction of the arsenic acid with HI is more than compensated for by the increase in oxygen required by the liberated formaldehyde-sulphoxylate group.

The formaldehyde-sulphoxylate group attached to the amino group in neoarsphenamine is a strong reducing agent, since it quantitatively reduces methylene blue and is itself oxidized to the corresponding bisulphite group, as will be

(I)



(II)



(III)

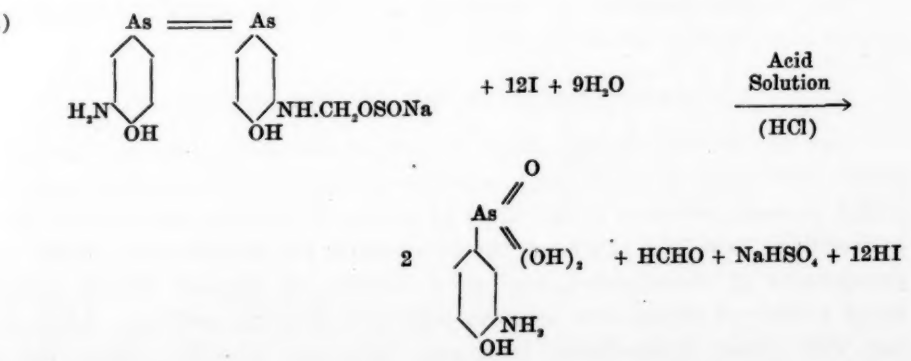


TABLE III

EFFECT OF ACIDITY ON TITRATIONS OF NEOARSPHENAMINES WITH IODINE

(A) HYDROCHLORIC ACID

AMT. OF SAMPLE GM.	AMOUNT OF ACID ADDED	% ACIDITY OF SOLUTION	% FINAL ACIDITY AFTER ADDITION OF I SOLUTION	C.C. N/10 I REQUIRED PER 100 MG. NEO.	MG. OXYGEN EQUIVALENT PER 100 MG. NEO.	MG. OXYGEN TO BE COMPENSATED BY REVERSE REACTION*	TOTAL ADJUSTED OXYGEN REQUIRED	INCREASE IN MG. OXYGEN
0.2	–	–	–	17.40	13.92	–	13.92	–
0.2	1.0 c.c. 18%	0.6	0.24	17.47	13.97	–	13.97	0.05
0.2	2.0 " "	1.12	0.45	17.57	14.05	0.21	14.26	0.34
0.2	5.0 " "	2.57	1.12	17.67	14.13	0.34	14.47	0.55
0.2	7.0 " "	3.40	1.54	17.70	14.16	0.57	14.73	0.81
0.2	10.0 " "	4.50	2.10	17.73	14.19	0.80	14.99	1.07
0.2	15.0 " "	6.0	3.00	17.79	14.22	1.00	15.22	1.30
0.2	20.0 " "	7.2	3.80	17.93	14.34	1.50	15.84	1.92
0.2	20.0 " 30%	12.0	6.32	No end Pt.				

(B) ACETIC ACID

0.2	20.0 c.c. 10%	5.0	2.5	17.45	13.96			
0.2	20.0 " 30%	12.0	6.0	17.32	13.85			
0.2	20.0 " 100%	40.0	20.0	16.80	13.44			
0.2	30.0 " "	50.0	25.0	16.47	13.18			

NOTE: In the above experiments, the 0.2 gram sample was dissolved in 30 c.c. H_2O, and 45 c.c. Standard iodine solution were used in each determination, the excess iodine being titrated back with $Na_2S_2O_3$ solution after 3 minutes.

shown in a subsequent publication. Theoretically there should be no doubt that this same group is oxidized by as strong an oxidizing agent as iodine, even in neutral solution in the cold, and our experimental results prove this to be true. However, as a certain amount of HI is formed during the titration with iodine by the reduction of the iodine, it is impossible theoretically to carry out this titration in absolutely neutral solutions. Although the actual acidity due to the formation of the HI is slight, as shown in Table II, it causes a slight amount of splitting off of formaldehyde-sulphoxylic acid and a resulting increase in the oxygen requirement. The equation representing the above reactions may be written as shown on p. 535.

Acetic acid, however, has no effect on the N-formaldehyde-sulphoxylate linking, and only the arseno group is oxidized by iodine even in strong acetic acid solution. The same effect is thus obtained as in titration of arsphenamine.

The results obtained by titrations of neoarsphenamine in solutions of various acidities are tabulated below.

ACTION OF HYDROCHLORIC ACID ON NEOARSPHENAMINES

Our observations on the effect of hydrochloric acid alone on neoarsphenamines were made in an attempt to precipitate the free neosulphinic acid from cooled aqueous solutions of the drug by means of uniform amounts of diluted hydrochloric acid, and analyzing the precipitates for sulphur and arsenic. The precipitates of neosulphinic acid were filtered on weighed Gooch crucibles using a layer of cotton over asbestos pulp as a filtering medium. After washing with dilute hydrochloric acid, and then cold distilled water, the neo-

TABLE IV

ANALYTICAL DATA OF NEOSULPHINIC ACIDS OBTAINED BY PRECIPITATION WITH HCl

SAMPLE OF NEO-ARSPHEN-AMINE	AMT. OF SAMPLE	% AS IN SAMPLE	% S IN SAMPLE	WT. OF FREE NEOSUL-PHINIC ACID IN GMS.	RATIO OF NEOARS. TO SULPHINIC*	% AS IN NEOSUL-SULPHINIC ACID**	% S IN NEOSUL-SULPHINIC ACID†	AS TO S RATIO IN NEOSUL-SULPHINIC ACID‡	REMARKS
H	0.4	19.35	8.00	0.2275	1.75:1	31.11	7.04	4.43:1	Sample dissolved in 30 c.c. 10% NaCl sol. and precipitated by 2 c.c. 18% HCl. Final acidity = 1.2% HCl. H_2O washings contained As tested by Lehmann's method, but amount not calculated.
J	0.4	18.80	10.34	0.2434	1.64:1	30.11	7.19	4.19:1	Precipitation incomplete. Considerable As in filtrate.
K	0.4	18.87	10.04	0.1987	2.01:1	30.96	6.51	4.75:1	Precipitation incomplete; more acid required for complete precipitation.
L	0.4	20.12	8.87	0.2329	1.72:1	31.88	6.44	4.45:1	H_2O washings tested quantitatively contained 3.7 mg. As; = 11 mg. neosulphinic acid.
M	0.4	18.52	10.89	0.2025	1.97:1	32.89	7.50	4.39:1	Required 10 c.c. 18% HCl to precipitate completely neosulphinic acid. Final acidity = 4.5% HCl.
G	0.4	19.65	9.01	0.2175	1.84:1	33.20	6.55	5.06:1	3.0 c.c. HCl required for complete precipitation.

*The high ratio of neoarsphenamine to neosulphinic acid is due to fact that commercial neoarsphenamines contain only approximately 60 per cent actual organic arsenical component.

**The theoretical As in pure monosubstituted neosulphinic acid is 33.80 per cent.

†The theoretical S in pure monosubstituted neosulphinic acid is 7.22 per cent.

‡The theoretical As to S ratio in monosubstituted neosulphinic acid is 4.68:1.

TABLE V

ACTION OF STRONG HYDROCHLORIC ACID* ON PRECIPITATES OF NEOSULPHINIC ACID

NO.	AMT. OF SAMPLE OF NEO.	% AS IN SAMPLE	% S IN SAMPLE	WT. OF DRIED PRECIPITATE IN GM.	% AS IN PPT.	% S IN PPT.	TOTAL AS OF ORIG. SAMPLE IN MG.	TOTAL AS OF PPT. IN MG.	% OF TOTAL AS IN PPT.	TOTAL S OF ORIG. SAMPLE IN MG.	TOTAL S OF PPT. IN MG.	% OF TOTAL S IN PPT.	REMARKS
1	0.4 gm.	19.35	8.00	0.2141	33.80	1.99	77.3	72.5	93.8	32.0	4.20	13.12	
2	0.4 "	19.73	11.00	0.1977	30.70	2.72	78.8	60.6	77.0†	44.0	5.37	12.20	Ppt. washed with 8% HCl. 9.5% of total As in filtrate.
3	0.4 "	19.73	11.00	0.2077	32.78	2.42	78.8	67.9	86.2†	44.0	5.02	11.40	6.2% of total As found in filtrate.

*The final acidity of the above solutions was approximately 10 per cent.

†In 2 and 3, in which the precipitates were washed with 8 per cent hydrochloric acid, the filtrates were analysed for arsenic, and 9.5 per cent and 6.2 per cent of the total arsenic were found respectively. This accounts for the decrease in total As found in these precipitates.

sulphinic acids were dried in a special vacuum chamber at 60-80° C. to constant weight. This usually required about twenty-four hours.

The results obtained by the above method were not very satisfactory from the standpoint of a quantitative method of analysis. It was found that varying amounts of hydrochloric acid were required to precipitate different brands of neoarsphenamine; that the neosulphinic acid, whether precipitated by dilute or strong hydrochloric acid, had an appreciable solubility in water, alcohol and very dilute hydrochloric acid, but was insoluble in hydrochloric acid of ten or more per cent. A part of the precipitate which was partially soluble in water, was reprecipitated by addition of concentrated hydrochloric acid. The second precipitate showed all the usual characteristics of arsphenamine hydrochloride.

Although the results of the above method, which are given in Table IV, do not warrant our drawing any definite conclusions, they are of particular interest in that they show large variations in the ratios of neoarsphenamine to the free neosulphinic acids of the different commercial samples, and a fairly close relationship in the arsenic to sulphur ratios of the free neosulphinic acids.

Although the results already obtained and outlined in Tables III (A) and IV show that the neosulphinic acids obtained by precipitation of solutions of neoarsphenamine by hydrochloric acid are hydrolyzed by this acid, the amount of hydrolysis depending on the strength of the acid, we carried out two experiments which confirmed the results of this observation beyond a doubt.

The free neosulphinic acid of two different samples of neoarsphenamine were precipitated from one per cent aqueous solutions by addition of 20 c.c. concentrated HCl, and the precipitates were filtered and dried as previously described. The results are given in Table V.

Whereas the precipitates were found to contain from 86 per cent to 95 per cent of the total original arsenic, they contained only 11 per cent to 13 per cent of the total original sulphur. Theoretically, if neoarsphenamine were not hydrolyzed by the hydrochloric acid, the precipitate of neosulphinic acid should contain approximately 35 per cent of the original sulphur. It is evident from the high arsenic and low sulphur content that the precipitate consists chiefly of arsphenamine hydrochloride. This was definitely proved to be true by the following experiment.

Thirty-five grams of neoarsphenamine were dissolved in 350 c.c. water; 200 c.c. HCl were added, precipitating the free neosulphinic acid. The mixture was allowed to stand for three days with occasional shaking. At the end of the third day, the precipitate had completely redissolved and the solution was colored a cherry red. The solution was cooled in an ice bath and 20 per cent sodium hydroxide solution added until the precipitate which at first formed (salvarsan base) redissolved and the solution was alkaline. Carbon dioxide gas was then passed into the solution until complete precipitation was obtained. The grayish-yellow precipitate, which proved to be salvarsan base, was then converted into the hydrochloride and precipitated from ether as an impure arsphenamine.

SUMMARY

Previous methods described for the determination of the chemical composition of neoarsphenamine are either too involved to be of practical importance or are subject to considerable error, because of the fact that they are based on titrations of the drug with iodine in acid solutions and separation of the active organic arsenic constituents from the uncombined organic and inorganic sulphur compounds by precipitation of the former with hydrochloric acid. Organic trivalent arsenic compounds cannot be titrated successfully by iodine in the presence of excess mineral acid, because of a reversible reaction taking place during the titration, this reversible reaction being influenced by increase in acidity.

The combined formaldehyde sulphoxylate groups in neoarsphenamine are either oxidized by iodine in solutions of weak acidity to the corresponding formaldehyde bisulphite groups, or are progressively hydrolyzed in increasing strengths of acid and finally oxidized completely to formaldehyde and sulphuric acid. Neoarsphenamine cannot be precipitated from its solutions by means of hydrochloric acid with any degree of analytical exactness because of the tendency of the precipitated neosulphinic acid to undergo hydrolysis with the formation of arsphenamine hydrochloride and organic and inorganic sulphur compounds, thus increasing the sulphur content of the filtrates.

CONCLUSIONS

1. A reversible reaction takes place when arseno compounds are titrated with iodine in presence of a mineral acid, less iodine being required than is theoretically necessary. The extent of this reversible reaction is directly proportional to the increase in acidity of the titrated solution.

2. Organic acids also have a tendency to promote this reversible reaction, but to a much smaller degree than mineral acids.

3. The combined formaldehyde sulphoxylate group in neoarsphenamine is oxidized by iodine in neutral solution to the corresponding formaldehyde bisulphite group, requiring 2 atoms of iodine.

4. In presence of even dilute solutions of hydrochloric acid, the combined formaldehyde sulphoxylate group undergoes a certain amount of hydrolysis, thus requiring more than 2 atoms of iodine for this group. For complete oxidation of this group 4 atoms of iodine or 2 of oxygen are required.

5. Strong solutions of hydrochloric acid (10 per cent or over) will completely hydrolyze neoarsphenamine into arsphenamine and the decomposition products of formaldehyde sulphoxylate. Arsphenamine hydrochloride has actually been isolated from this reaction.

The author wishes to express his appreciation to Dr. A. E. Sherndal for his interest in this work, and for his helpful criticisms in the preparation of the manuscript.

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THE INFLUENCE OF MAGNESIUM SULPHATE ON THE EXPULSION OF BILE FROM THE GALL BLADDER*

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MAGNESIUM sulphate has been widely used during the past six years to cause expulsion of bile into the duodenum. Upon this alleged property of the salt rests its reputation in the drainage of the gall bladder by the Meltzer-Lyon method.[1, 2]

However the subsequent work of some clinicians who have applied this method in the treatment and diagnosis of gall bladder conditions and of several physiologists have not substantiated the original claims made for magnesium sulphate as a stimulator for the contraction of the gall gladder and relaxation of the sphincter of Oddi. (Max Einhorn,[3] Thos. R. Brown, Frazer,[4] J. W. McNee,[5] Bickel.[6]) The marked discrepancy in the views of prominent clinicians, coupled with the fact that one of us (W. H. G.) had had good clinical results (in diagnosis) from this method, led us to make several experiments in the hope of throwing additional light on the action of this salt when introduced into the duodenum.

In 1921 Bassler[14] could not see a contraction of the gall bladder nor obtain bile from the duodenum when magnesium sulphate was introduced through the duodenal tube in anesthetized patients, but the results of Matsuo[16] and of Silverman and Menville[15] are at variance with those of Bassler. Matsuo saw in the duodenum an appearance of dye which he injected into the gall bladder at the same time that magnesium sulphate was given, and Silverman and Menville using a fluoroscope and visualizing the gall bladder by the injection of phenoltetrabromphthalein into the blood, saw the gall bladder become smaller after the administration of $MgSO_4$. Friedenwald, Martindale, and Kearney[13] performed experiments which did not confirm the positive clinical reports, noted above.

In our experiments we tried to have the conditions as near to normal as possible, concerning both the physical condition of the dog and the nerve connections and the condition of the duodenum and of the papilla of Vater, as it is upon this part that the magnesium sulphate is supposed to act. For this reason we did not use the choledochus fistula operation of Prof. Pavlov[7] in which a section of the duodenum surrounding the papilla is excised and sutured to the skin; nor the double biliary fistula as performed by one of us (G.v.V.) previously.[8] We selected the gall bladder fistula of Schiff,[9] because the connections of the papilla of Vater are not disturbed, and the choledochus is pre-

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served intact in this operation. One of us has shown[10] that in a dog with such a fistula, the beginning of the expulsion of bile into the duodenum can be clearly recognized, that the expulsion of bile through the gall bladder is at the same time inhibited, and that within ten to fifteen minutes after the bile begins to flow into the duodenum, there appears in the gall bladder a pure colorless mucus, without a suggestion of bile. With the cessation of the flow of bile into the duodenum, i.e., at the end of the period of digestion, bile reappears in the gall bladder, and the mucus flowing from the gall bladder fistula becomes suddenly colored.

In addition to the gall bladder fistula of Schiff we performed in our dogs a lateral duodenal fistula, according to Pavlov.[7] Without disturbing the continuity of the duodenum, it was sutured to the surface of the abdomen, so that the opening in the duodenum was about 7 to 9 cm. below the papilla of Vater. This operation is to a certain degree a substitute for the choledochus fistula in the double fistula operation.[8] The arrangement of the duodenal fistula (close to the papilla of Vater) permits us to introduce the magnesium sulphate directly into the duodenum, as was done by Meltzer,[1] so that the possibility of the action on the biliary apparatus is insured. And though we cannot quantitatively determine the amount of bile in the duodenum, we can make certain at any moment whether it is flowing into the duodenum, for we can at any time obtain a specimen of the duodenal contents through this fistula.

The following experiments in dogs were not carried out until a month had elapsed after the operation, and we had assured ourselves that the condition of the animals and of the biliary secretions were normal. The experiments were performed in the fasting condition, fifteen to twenty-four hours after the last feeding, and according to the general conditions of experimentation in Pavlov's laboratories.[7]

Fifty c.c. of a 25 to 33 per cent solution of magnesium sulphate at a temperature of 35-40° C. was introduced into the duodenal fistula, and after ten to fifteen minutes, the duodenal fistula was opened and the solution allowed to run out.

We append here protocols from two of our dogs. Table I shows the influence of magnesium sulphate upon the volume of bile secreted (the fluid from each fistula being collected and measured every fifteen minutes). Table II shows the influence of magnesium sulphate on the pressure of bile in the biliary system.

The objection can be raised that conditions are not normal when there is a gall bladder fistula because the bile continuously flowing out of the fistula may prevent a filling of the gall bladder and ducts, and secondly in such a case the magnesium sulphate could not exert its influence to cause expulsion of the bile into the duodenum, because the gall bladder would contract to force the bile along the line of least resistance, i.e., through the wide fistula instead of along the smaller cystic duct. Although this objection was partly refuted through an earlier experiment by one of us,[8] we devised here a special arrangement of the apparatus to prevent the flow of bile from the gall bladder fistula and to maintain a constant and normal pressure in the biliary system.

A schematic representation of the operation and apparatus is shown in Fig. 1.

TABLE I

DOG 1, "JACK". APRIL 10, 1923

TIME	GALL BLADDER FISTULA		DUODENAL FISTULA
11:15			
11:30	2.8 c.c.	All specimens = pure bile.	Little or no flow into the duodenum.
12:45	2.2		
12:00	2.6	Amt. secreted for 1st hr. (12:00-	
11:45	0.9	1:00) = 4.6 c.c.	Reaction weakly acid.
12:30	0.5		
12:15	0.6		
12:50§		§Introduction of 20.0 c.c. 30% $MgSO_4$ through the duodenal fistula, and allowed to remain 10 min.	
1:05	1.8	All specimens pure bile as for-	Fistula opened at 12:40 P. M.: 5.0 c.c.
1:20	0.8	merly.	clear fluid, without bile obtained; reac-
1:35	1.2		tion weakly acid.
1:50	0.6	Amt. excreted for 2nd hr. (1:05-	
2:05	1.0	2:05) = 4.4 c.c.	
		During the whole experiment there was practically no flow into the duodenum.	

DOG 1, "JACK". APRIL 13, 1923

TIME	GALL BLADDER FISTULA		DUODENAL FISTULA
9:45			
10:00	0.4 c.c.	Pure mucus.	
10:07		Pure bile begins to flow and con-	
10:15	4.8	tinues till end of experiment.	
10:30	4.2		
10:45	4.6	Amt. secreted for 1st hour.	
11:00	2.8	(10:00-11:00) = 17.4 c.c.	
11:00§		§Introduction 50.0 c.c. 30% $MgSO_4$ into the duodenal fistula and allowed to remain 10 min.	
11:10			Duodenal fistula opened and a few drops
11:15	3.1	All specimens pure bile.	of clear fluid without bile obtained.
11:30	2.2		After 11:00 A. M. there was a flow of
11:45	1.71	Amt. excreted for the hr.	clear fluid without bile; reaction neutral.
12:00	1.1	(11:00-12:00) = 8.1 c.c.	
12:15	1.1		

DOG 1, "JACK". APRIL 14, 1923

TIME	GALL BLADDER FISTULA		DUODENAL FISTULA	
12:00				
12:15	1.6 c.c.	Secreted ¾ hr. (12:45-1:15) =	24.0 c.c.	Weakly colored bile.
12:30	2.0	3.8 c.c.	16.0	Reaction acid; no bile.
12:45	2.0		8.0	Idem.
1:00	0.8		4.5	"
1:15	1.0		6.00	Reaction acid; bile colored.
1:15§		§Introduction 50.0 c.c. 25% $MgSO_4$ in duodenal fistula, and allowed to remain 10 minutes.		
1:30	2.3	Secreted for ¾ hr. (1:15-2:00)	8.0 c.c.	Reaction acid; no bile.
1:45	1.2	= 4.5 through g. b. fist.	30.0	Idem. " "
2:00	1.0		95.0	" " "

DOG 1, "JACK". APRIL 20, 1923

TIME	GALL BLADDER FISTULA		DUODENAL FISTULA	
10:30				
10:40	1 drop mucus.			Fistula opened; slight amt. bile.
10:45	0.8 c.c.		3.0 c.c.	
11:00		Amt. excreted through the g. b.		Reaction neutral; strongly bile
11:15	2.0	fist. for the hr. (11:00-12:00)	3.2	colored.
11:30	2.2	= 6.6 pure bile.	11.0	Neutral no bile.
11:45	1.6			" " "
11:45§		§Introduction 50.0 c.c. 25% $MgSO_4$ through duo. fistula and allowed to remain 15 min.		
12:00	1.4		18.5 c.c.	Clear bile free fluid; reaction
12:15	1.4		3.0	neutral.
12:30	1.0		1.0	Reaction alkaline; bile free, for
12:45	1.1		0.5	all specimens till end of exp.
1:00	1.1		0.5	
1:15	1.1		0.5	

TABLE I—CONT'D

DOG 2, "LORA". FEBRUARY 6, 1924

TIME	GALL BLADDER FISTULA		DUODENAL FISTULA	
5:30				
5:45	0.6 c.c.	All specimens pure bile. Amt.		
6:00	0.4	from g. b. for the hr. (5:30-		
6:15	0.2	6:30) = 1.5 c.c.		
6:30	0.3			
6:30§		§Introduction 50 c.c. 30% $MgSO_4$ through duo. fistula and allowed to run out after 5 min. At 6:35 fistula was opened for $MgSO_4$ to drain out. At 6:40		
6:40		some alkaline flow of mucus from the duodenal fistula.		
6:45	0.8		20.0 c.c.	Bile free fluid, mucoid, all specimens, to end of experiment.
7:00	0.4	All specimens pure bile. Amt.	0.5	
7:15	0.3	from the g. b. fist. for the hr.	0.5	
7:30	0.0	(6:30-7:30) = 1.5 c.c.	0.0	

TABLE II

EXPERIMENTS ON THE PRESSURE OF BILE IN THE BILIARY SYSTEM, MEASURED IN MM. OF BILE. SEE FIG. 1.

DOG 1, "JACK". APRIL 15, 1924

TIME	BILE PRESSURE IN BILIARY SYSTEM	DUODENAL FISTULA
11:45		
12:00	17. mm. Rises gradually to 25. mm.	Several drops bile-free fluid.
12:15	25.	
12:15§	§Introduction 50.0 c.c. 30% $MgSO_4$ into the duodenum through the duo. fistula.	
12:30	24-38 Sinks to 24 mm., and then	
12:45	46. rises gradually to 38 mm.;	Fistula opened; 10 c.c. of a cloudy alkaline fluid obtained; bile-free.
1:00	51. then rises gradually to 46 mm. and becomes stationary at 51 mm.	At 1:00 P. M. several drops of a clear alkaline, bile-free fluid.

DOG 2, "LORA". FEBRUARY 19, 1924

TIME	BILE PRESSURE IN BILIARY SYSTEM	DUODENAL FISTULA
11:50	50. mm.	
12:10	50-56 Rises from 50 to 56, and in	4.0 c.c. Clear, alkaline, bile-free.
12:25	72-74 last min. to 72. Varies between 72 and 74.	Several drops, " " "
12:25§	§Introduction 50 c.c. 30% $MgSO_4$ through duo. fist. and after 7 min. duodenal fist. opened.	
12:40	78-80 Varies 78-80.	13.5 c.c. Cloudy, bile-free fluid.
12:55	80-82 " 80-82.	18.5 Clear, alk., " " "

DOG 2, "LORA". FEBRUARY 11, 1924

TIME	BILE PRESSURE IN BILIARY SYSTEM	DUODENAL FISTULA
5:00	22 mm.	
5:15	22-24	Several drops of mucus.
5:30	24-28	" " clear, bile-free fluid.
5:30§	§Introduction 70 c.c. 30% $MgSO_4$ through the duodenal fistula; fist. opened in 15 min.	
5:45	28-36	Reaction alkaline; bile-free.
6:00	36-46	" " " "
6:15	44.	" " " "

DOG 2, "LORA". FEBRUARY 13, 1924

TIME	BILE PRESSURE IN BILIARY SYSTEM	DUODENAL FISTULA
5:30	80.00 mm.	
5:45	80-84	9.0 c.c. Clear, bile-free fluid.
5:45§	§Introduction 40 c.c. 25% $MgSO_4$ through the duodenal fistula; fistula opened after 10 min.	
6:00	84-80	10.0 c.c. Neutral, bile-free fluid.
6:15	82-84	1.0 Alkaline, bile-free.
6:30	80	1.0 " " "

The cannula of the gall bladder fistula was closed with a cork containing a glass rod of 4 mm. diameter, and a rubber tube was used to connect the cannula with an ordinary burette. The latter was filled with fresh bile collected from the same dog, and the burette was adjusted so that the height of the fluid therein stood slightly higher than the inner opening of the gall bladder fistula. (See Fig. 1.) Consequently the gall bladder and biliary ducts were kept filled with bile, and a normal pressure maintained—the same that would be in a normal animal without a fistula. Every new portion of the bile coming from the gall bladder fistula must now be forced out against the pressure of the column of bile in the burette, and consequently the pressure in the whole biliary system will be raised. This pressure can be controlled and measured by the height of the column of bile in the burette above the level of the inner end of the tube in the gall bladder fistula. We are thus able to prevent the abnormally low pressure which would otherwise result from the escape of bile

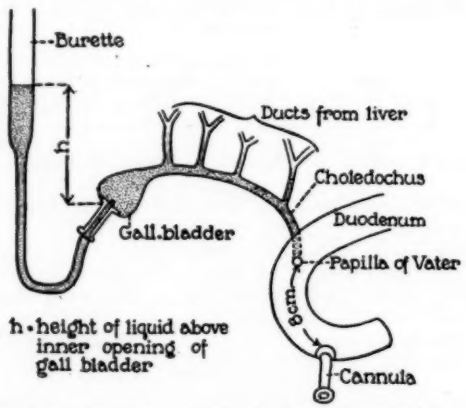

Fig. 1.—Schematic representation of operation.

from the gall bladder fistula; and by opening the duodenal fistula we can ascertain at any moment whether bile is entering the duodenum.

Table II represents the observations on the change in pressure resulting from the introduction of magnesium sulphate into the duodenum, and observations on the appearance of bile in the duodenum. This salt had no constant effect on the pressure of bile in the gall bladder and ducts, and in not a single case did we see an expulsion of bile into the duodenum as a result of the action of the magnesium sulphate.

When comparing the specimens of bile secreted before and after the introduction of the magnesium sulphate, we could not perceive in our dogs the effect on the color of bile, as noted by Einhorn in patients.[3] Our experiments here cannot be considered as giving conclusive evidence on the secretion of bile after $MgSO_4$, because secondary influences such as the acid secretion of the peptic glands and the expulsion of the stomach contents into the duodenum were not absolutely ruled out, but they agree with the results of Nissen and Jordan.[11, 12] The effect of food was, however, eliminated, as the dog was fasted for twenty-four hours preceding the experiment.

Just after performing each experiment, in order to convince ourselves that the secretory and motor functions of the biliary apparatus were working

normally, we tested them through the application of their normal stimulators, viz., through the introduction of HCl into the duodenum or through the feeding of milk. In the former case we noted a copious outflow of bile through the bladder fistula, as a sign of the increased secretion; and with milk, bile appeared promptly in the duodenum.

DISCUSSION

In the hands of many eminent clinicians (Friedenwald, Smithies, J. Meakins, Langdon Brown) duodenal drainage by means of magnesium sulphate has proved of value. The discrepancy between our experimental results and the clinical findings may be another one of those cases in which the clinical method is in the empiric stage. It is also possible that the physiology of the dog here differs from that of the human. There is an anatomic difference because the dog has two pancreatic ducts, one of which does not open at the papilla of Vater. We intend to repeat our experiments after ligating that pancreatic duct which does not open at the papilla of Vater. One of us (W. H. G.) has shown clinically that magnesium sulphate introduced into the duodenum of the human causes an excretion of pancreatic juice equal in enzyme activity to the secretion which follows the administration of beef extract; and it may be this flow from the pancreas which causes the relaxation of Oddi's sphincter.

Our work is in agreement with that of Friedenwald and Martindale[13] on dogs; but it does not agree with that of Meltzer, upon which the clinical method is based, and there is certainly room for doubting the ordinary explanation which has been given of the physiology of duodenal drainage, as suggested in Meltzer's footnote in 1917.

SUMMARY

In our experiments with dogs, using a special device for maintaining normal pressure in the bile ways, we found absolutely no influence of magnesium sulphate on the expulsion of bile into the duodenum.

We desire to thank Prof. Pavlov, in whose laboratory these experiments were performed, for his advice and general supervision of our work.

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VARIATIONS IN THE BLOOD-SUGAR CONTENT FOLLOWING THE ADMINISTRATION OF INSULIN*

By Henry J. John, M.D., Cleveland Clinic, Cleveland, Ohio

PROLONGED and repeated observations of the effects of the intravenous injection of insulin on blood-sugar content have shown unexpected and marked variations not only in different patients, but even in the same patient after repeated doses. These variations have been so contrary to my own preconception of the action of insulin that it has seemed worth while to present the observations in a group of individual cases. It is certainly important to determine if possible upon what factors the individual response to insulin depends; and this can only be determined by accumulated experience in large numbers of cases.

Graphic charts present the best material for comparative study, and I am therefore presenting two series of charts, one showing the effects of repeated doses of insulin in 20 patients during a period of seven hours (Charts 1 and 2), each of the other nine charts showing the effects of insulin in a single patient on two or more days (Charts 3 to 11).[1]

In every case the observation period started at about 8:30 A.M. (indicated as 0 hr.), the first dose of insulin being followed by breakfast; lunch was taken at 11:30 A.M.—3 hours; and dinner at 4:30 P.M.—7 hours. The diet was uniform, excepting when otherwise noted on the chart, and consisted of 100 gm. of carbohydrate, 60 gm. protein, 128 gm. fat, 1800 calories. The doses of insulin varied from 3 to 60 units. In interpreting the charts, the effect of the meals on the blood sugar must be taken into consideration as they are always followed by a physiologic postprandial hyperglycemia, which with but few exceptions is exaggerated in diabetes.

A study of the 20 individual curves included in Charts 1 and 2 will show that in all but four (117672, 130990, 114782, 136108) an immediate drop in the blood-sugar content followed closely after the primary dose of insulin. With but one exception the drop is moderate but in that one case—133929—the drop amounted to 281 mg./100 c.c. during the first hour.

In one of the four cases, in which an actual rise of the blood sugar occurred immediately after the administration of the primary dose of 20 units of insulin, the rise continued even after the administration of 10 more units of insulin two hours later.

Chart 3 is that of a fairly severe case of diabetes in a young man twenty-six years of age. On April 13 following the afternoon administration of 16

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[1]*Explanation of the charts*: The heavy lines represent the successive changes in the blood-sugar level, as indicated by the measurements made at the points specified by the heavy dots intersecting the lines. The dosage of insulin in units—and the time of administration—is indicated by the numbers in the circles. The blood sugar scale—in mg. per 100 c.c.—is indicated at the left margin. The blood sugar estimations were made with the Myers' modification of the Benedict method.

units of insulin, there was a steady drop of the blood sugar which was estimated every fifteen minutes for the next three hours, 30 units of insulin given an hour later being followed by a much less marked drop down to 52-56 mg./100 c.c. where the blood-sugar level was maintained with only a slight reaction forty-five minutes after the second dose. On April 24, however, the afternoon dose of 15 units was followed by a rise of the blood sugar as indicated on the chart.

Chart 4 is that of a young woman twenty-five years of age who was brought to the hospital in coma. This picture therefore is not complicated by the taking of any food. As is shown by the chart, on August 21 following the first dose of 7 units of insulin, the blood sugar rose for two hours and twenty

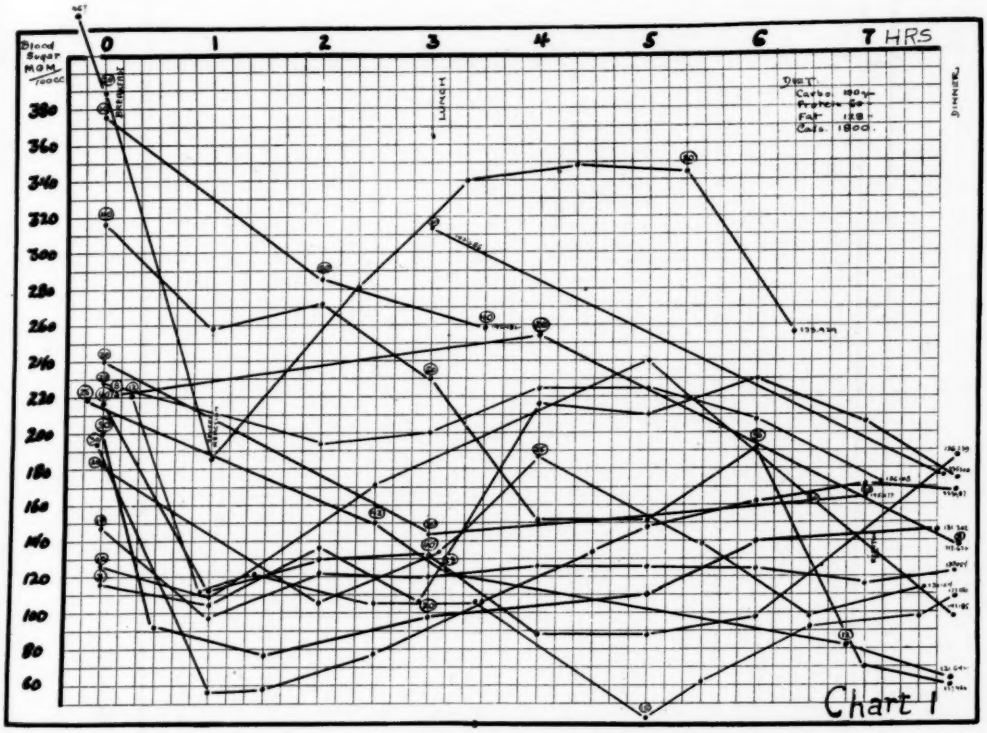

Chart 1.—Blood-sugar curves showing variations in the response to varying doses of insulin in sixteen diabetic patients.

minutes, when the administration of 15 more units was followed by a moderate decrease of the blood sugar which was progressive for four hours, when the administration of 16 more units was followed by a more marked drop. Two days later the administration of the morning dose of insulin—15 units—was followed by a drop of the blood sugar from 297 to 232 mg./100 c.c. at the end of three hours; 15 more units were then given following which the blood sugar rose to 375 mg./100 c.c.; when 26 more units of insulin reduced the blood sugar to 263 mg./100 c.c. in three hours and twenty minutes.

Chart 5 is that of a woman thirty-nine years of age, a severe diabetic. On August 28, 1924, the administration of 25 units of insulin in the morning was followed by a rise of blood sugar from 300 to 348 mg./100 c.c., when 25 units more were administered following which there was a steady and continued drop

of the blood sugar to 141 mg./100 c.c. On February 3, 1925, the blood-sugar level in this same patient was 384 mg./100 c.c. At this time the administration of 25 units of insulin was followed in one and one-half hours by a drop to

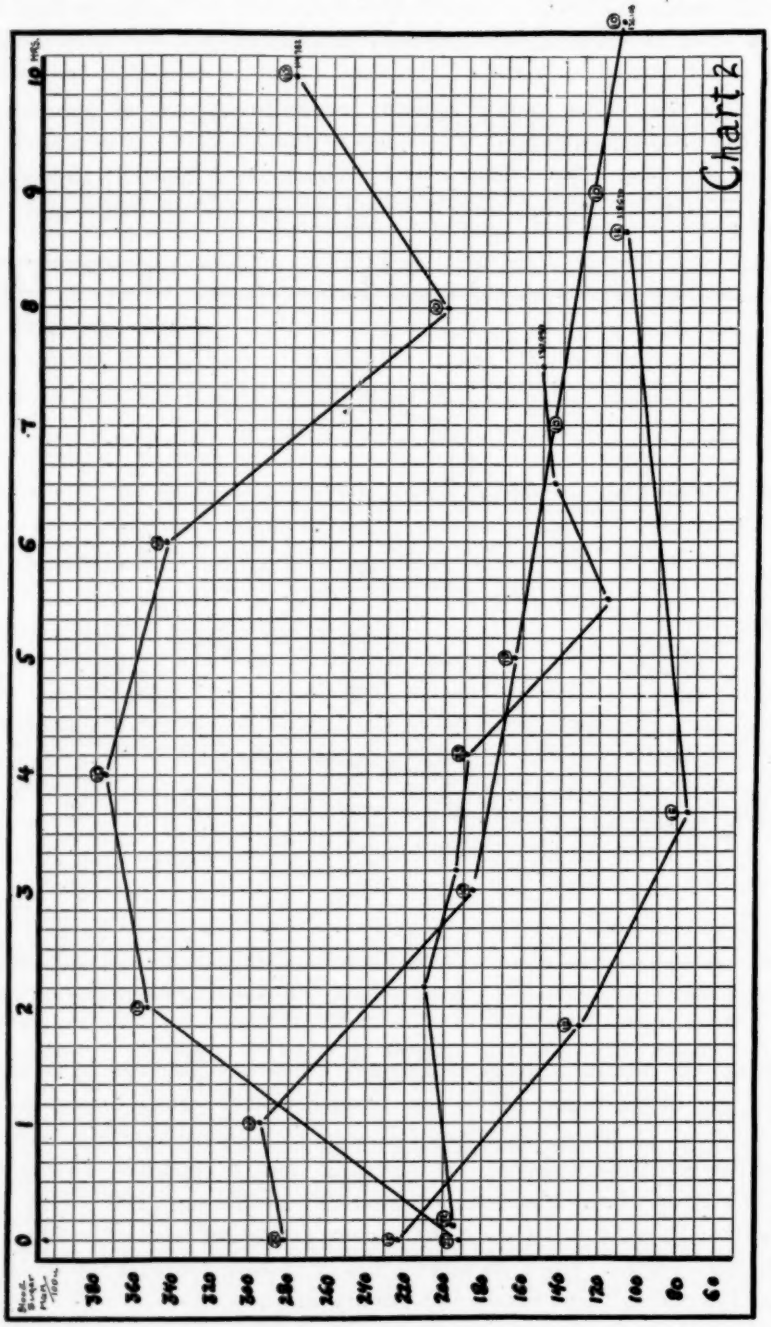

Chart 2.—Blood-sugar curves showing variations in the response to varying doses of insulin in four diabetic patients.

303 mg./100 c.c.; after this during the next two hours the blood sugar rose to 336 mg./100 c.c. when again 25 units of insulin were given, with a resultant drop during the next two and one-half hours to 171 mg./100 c.c. At this point

the blood sugar again began to rise reaching 275 mg./100 c.c. in two hours. One might be tempted to explain the rise in the second curve by the assumption that the insulin effect wore out at the end of two and one-half hours; but how can one explain the primary rise in the first curve? One might assume that the primary dose of insulin was not sufficient to counteract the postprandial

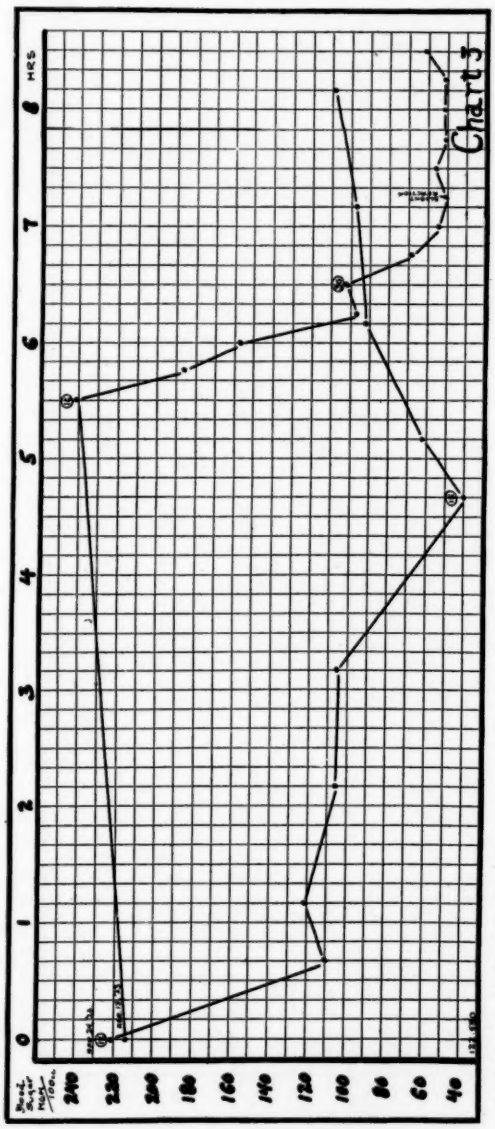

Chart 3.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on two different days.

hyperglycemia—but certainly 25 units of insulin should be sufficient to counteract the 20 grams of carbohydrate included in the food taken at breakfast.

Chart 6 is that of a man twenty-seven years of age. On November 18, 1924, a rise in the blood sugar from 190 to 263 mg./100 c.c. followed breakfast. One hour after breakfast the administration of 10 units of insulin was followed by a steady fall of the blood sugar to 105 mg./100 c.c., six hours later, when the

patient had a questionable reaction. Two days later, on November 20, 10 units of insulin were given at noon, when the blood sugar was 146 mg./100 c.c., following which during the next four hours there was a steady drop to 112 mg./

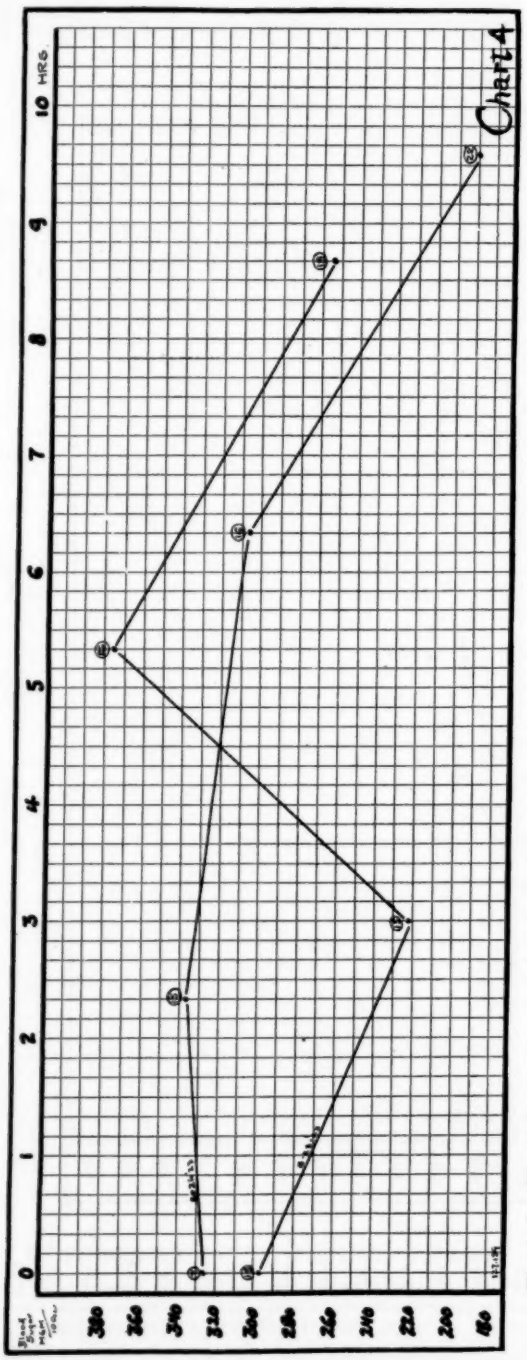

Chart 4.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on two different days.

100 c.c., the progressive decrease in the blood sugar being uninterrupted by luncheon, which was taken immediately after the dose of insulin was given. In contrast to these two curves is that of the following day when with a fasting

blood sugar of 92 mg./100 c.c. the administration of 10 units of insulin was followed by a rise throughout the day to 217 mg./100 c.c. seven hours later.

Chart 7 gives observations during nine successive days on a young man twenty-six years of age who was brought to the hospital in coma. On admission the CO_2 tension in the blood was 21.4 volume per cent; there was heavy acetone in the plasma, and marked hyperglycemia. On the morning of February 14,

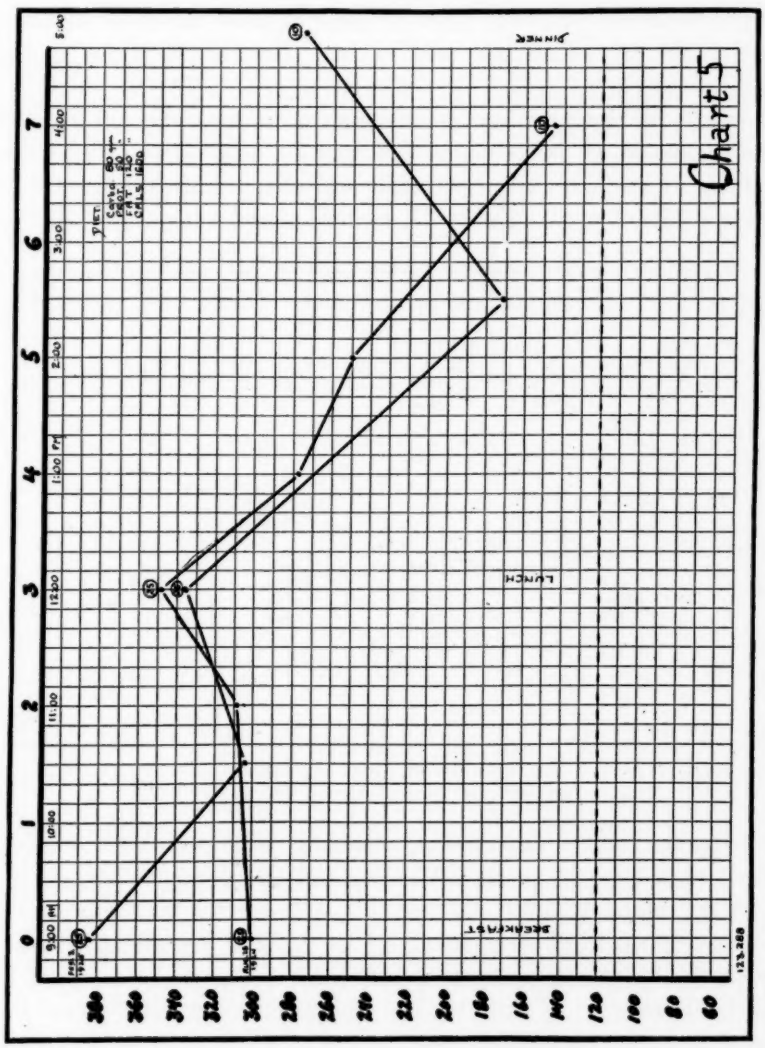

Chart 5.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on two different days.

1925, following the administration of 20 units of insulin, the blood sugar dropped from 280 to 256 mg./100 c.c. in an hour and ten minutes, when 40 more units of insulin were administered together with the intravenous injection of 250 c.c. of a 10 per cent glucose solution. This was followed by an increase of the blood sugar to 316 mg./100 c.c. during the next hour and a half, when another dose of 40 units of insulin together with 250 c.c. of the 10 per cent glucose solution was followed by a further rise to 352 mg./100 c.c. Again 40 units of insulin with another 250 c.c. of 10 per cent glucose solution were given and

two hours later 20 units of insulin were given hypodermically with a resultant decrease of the blood sugar during the next six hours to 120 mg./100 c.c., when 40 units of insulin with glucose solution were again given with no resultant reaction. On the following day, February 15, the patient started with a blood sugar content of 442 mg./100 c.c.; as indicated by the chart repeated doses of insulin were required to reduce the blood sugar to 187 mg./100 c.c. at the end of six hours with later decreases after two more successive doses to 53 mg./100 c.c. It is of interest to note that although two of these doses of insulin were combined with 250 c.c. of 10 per cent glucose, the fall in blood sugar was continuous.

On February 16, the morning blood-sugar level was only 200 mg./100 c.c. The administration of 40 units of insulin was followed by a slight drop to 172 mg./100 c.c. Two hours later, when 40 units of insulin together with 250 c.c. of glucose solution were given with a subsequent very slight rise during

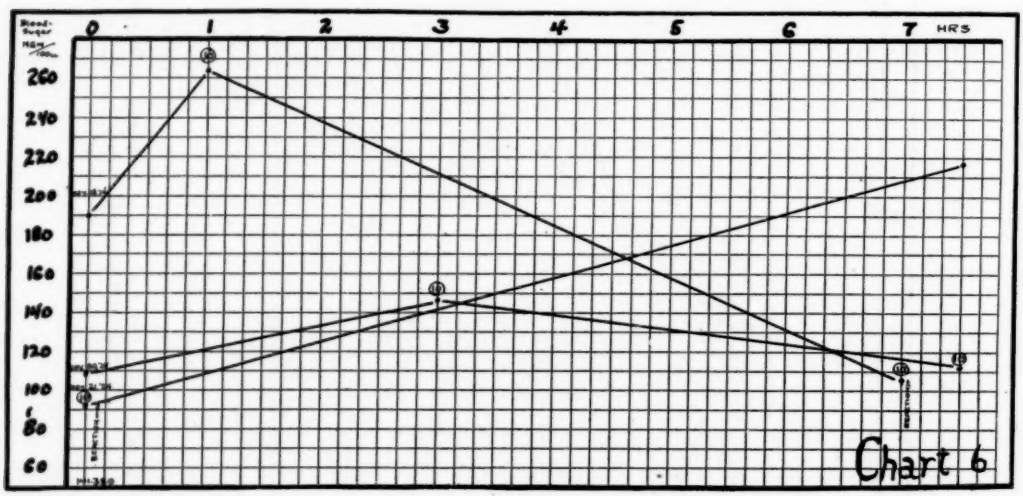

Chart 6.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on three different days.

the next two hours to 175 mg./100 c.c. Twenty units of insulin at this point were followed by a drop to 85 mg./100 c.c.; and two further doses of 20 units each carried the blood sugar to 67 mg./100 c.c. during the next four hours.

The curves of the following days give us further graphic pictures of the variability of the blood-sugar changes following the administration of insulin. The curves of February 18 and 19 are especially interesting because of the small doses of insulin which were given.

Chart 8 is that of a young man thirty-five years of age, with severe diabetes, and generalized xanthoma diabeticorum.

Chart 9 is that of a woman thirty-four years of age who was brought to the hospital in coma. In each of these the progress of events is sufficiently noted by the charts.

Chart 10 gives graphically the observations on seven successive days in a very severe case of diabetes in a woman thirty-eight years of age. The individual curves in this case show a considerable variation. The food was limited

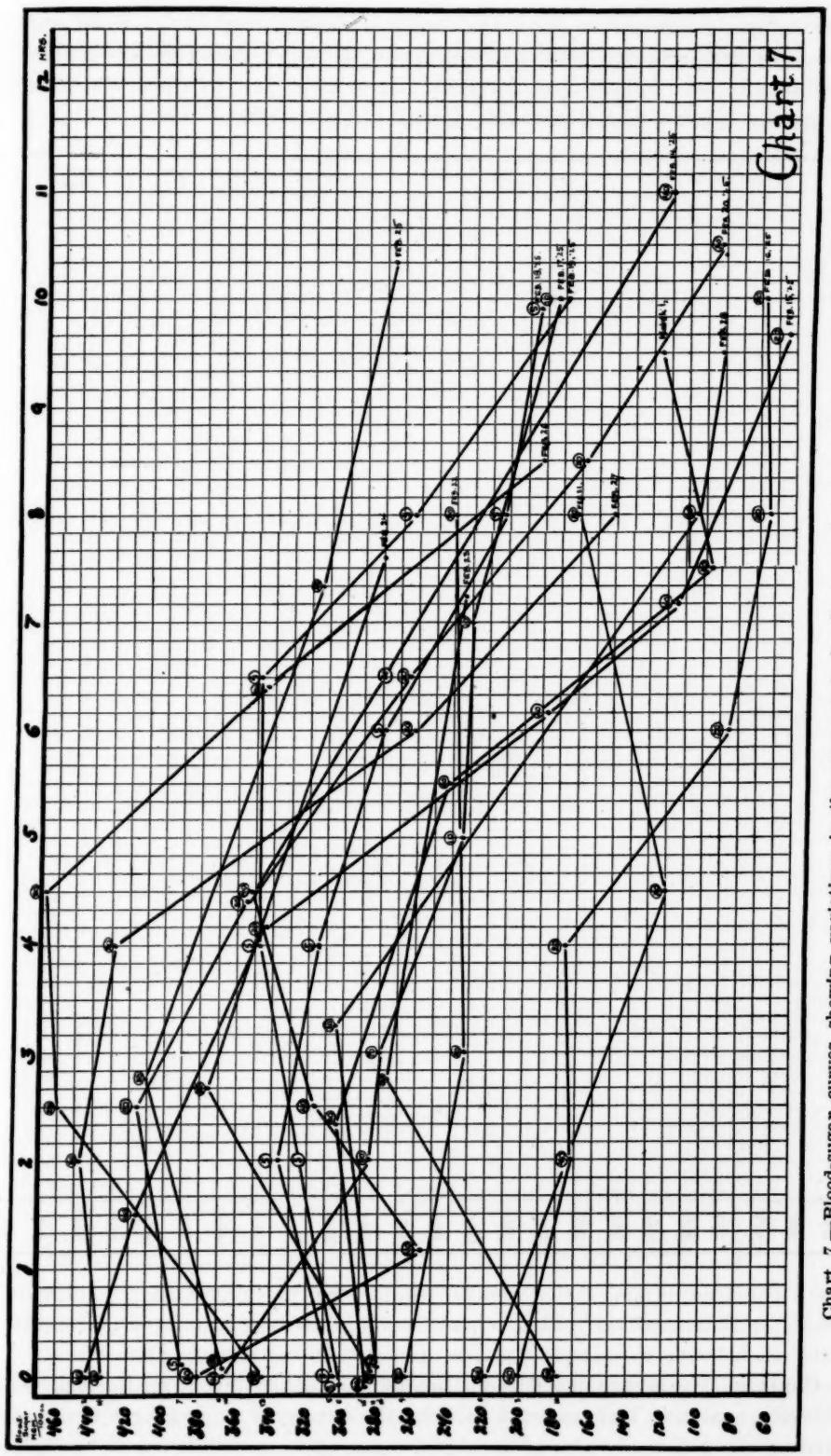

Chart 7.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on nine successive days.

to practically a maintenance diet. On four mornings a drop in the blood sugar followed the first administration of insulin; on the other mornings the primary dose of insulin was followed by a rise in the blood sugar. In every instance, however, the blood sugar decreased after the dose given at noon. The more

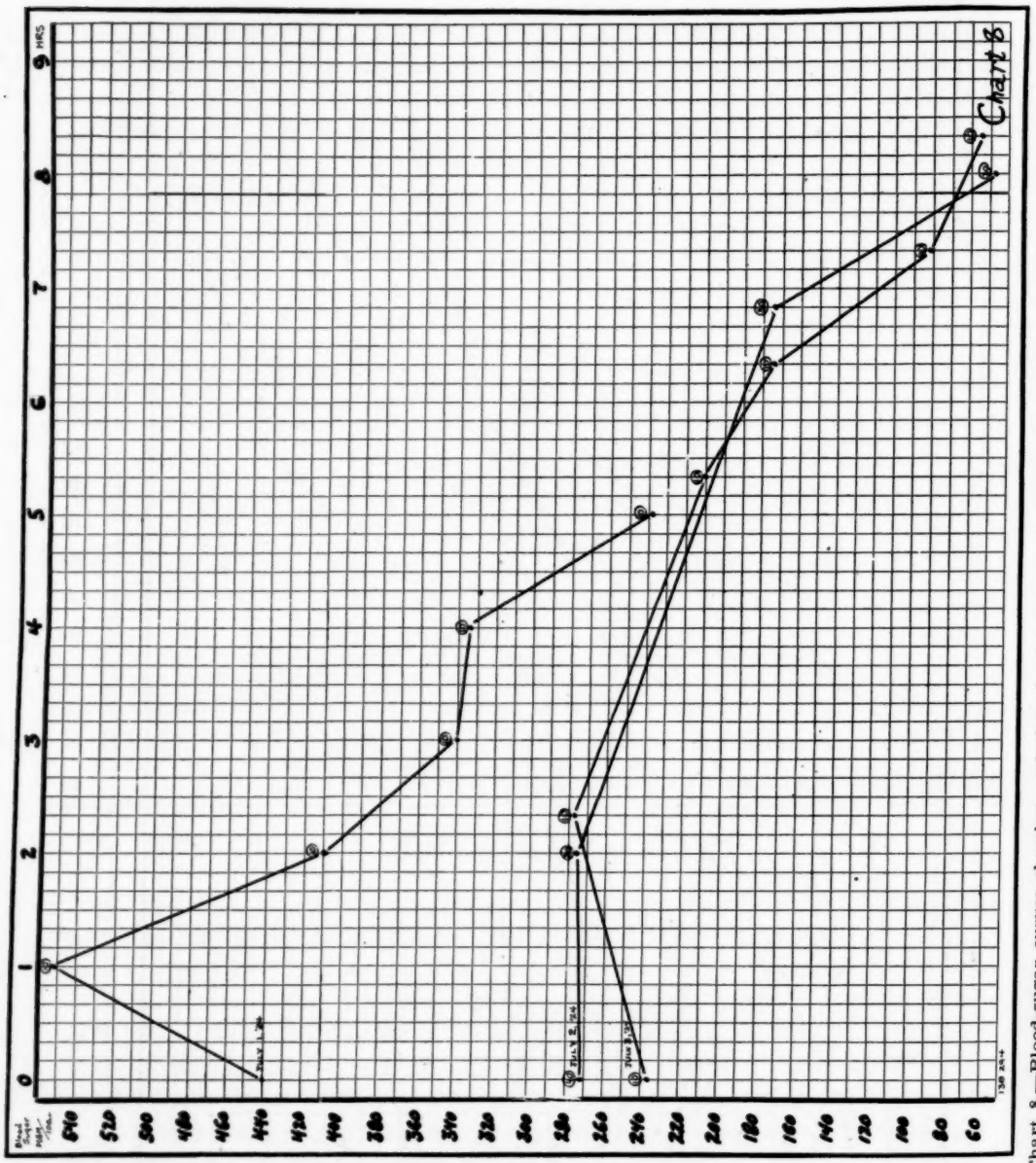

Chart 8.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on three successive days.

marked response during the afternoon would seem to suggest that insulin may have a cumulative action. This, however, would not explain the apparent lack of relation between the size of the dose and the degree of response.

Chart 11 is presented as it seems to summarize well the indications of those previously analyzed, and presents in brief the extent of our present knowledge regarding the practical value of insulin in cases of severe diabetes. The patient

was a woman forty-eight years of age who was in coma when I first saw her. Hourly doses of insulin were at once instituted and hourly estimations of the blood sugar were made throughout two successive days.

Although the insulin dosage was uniform and given at regular intervals,

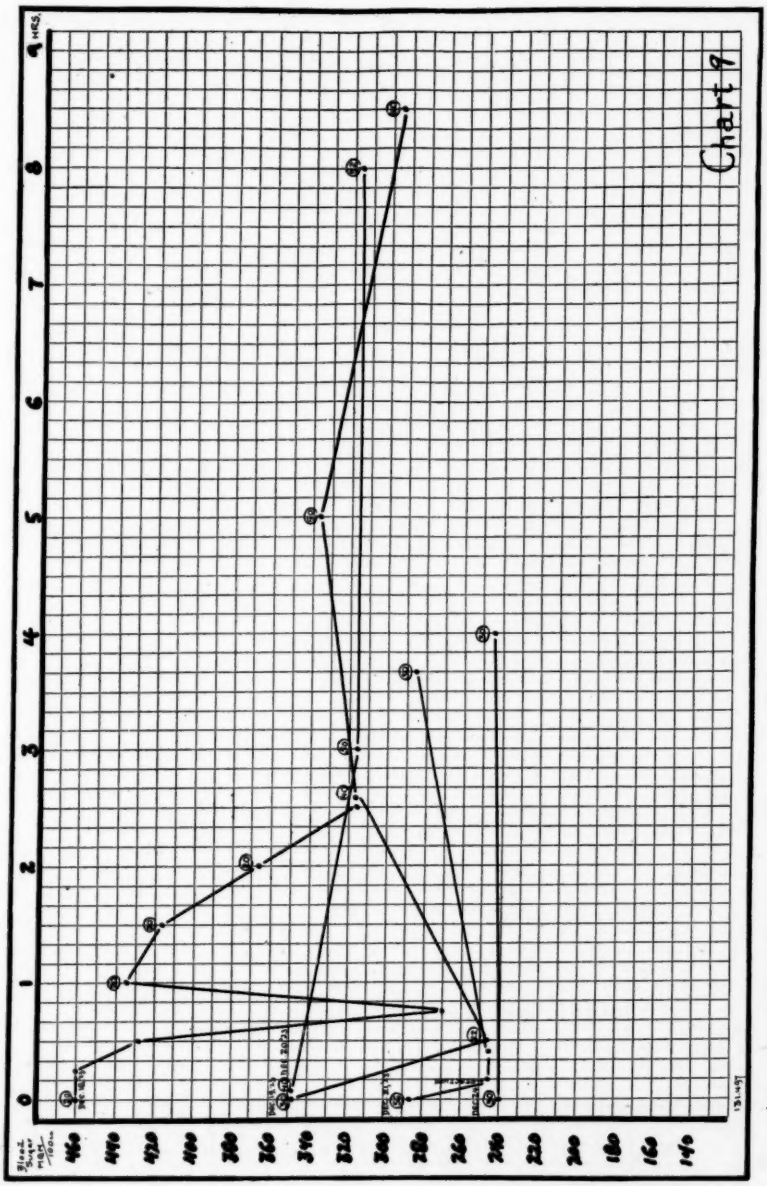

Chart 9.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on five different days.

the response during the first nine hours was not uniform. Nevertheless, during the first day with the exception of the temporary slight increases of the blood sugar there was a fairly uniform drop to below the normal level; and during the second day, this low level was maintained by means of the administration of 20 units of insulin at regular intervals.

Chart 8.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on three successive days.

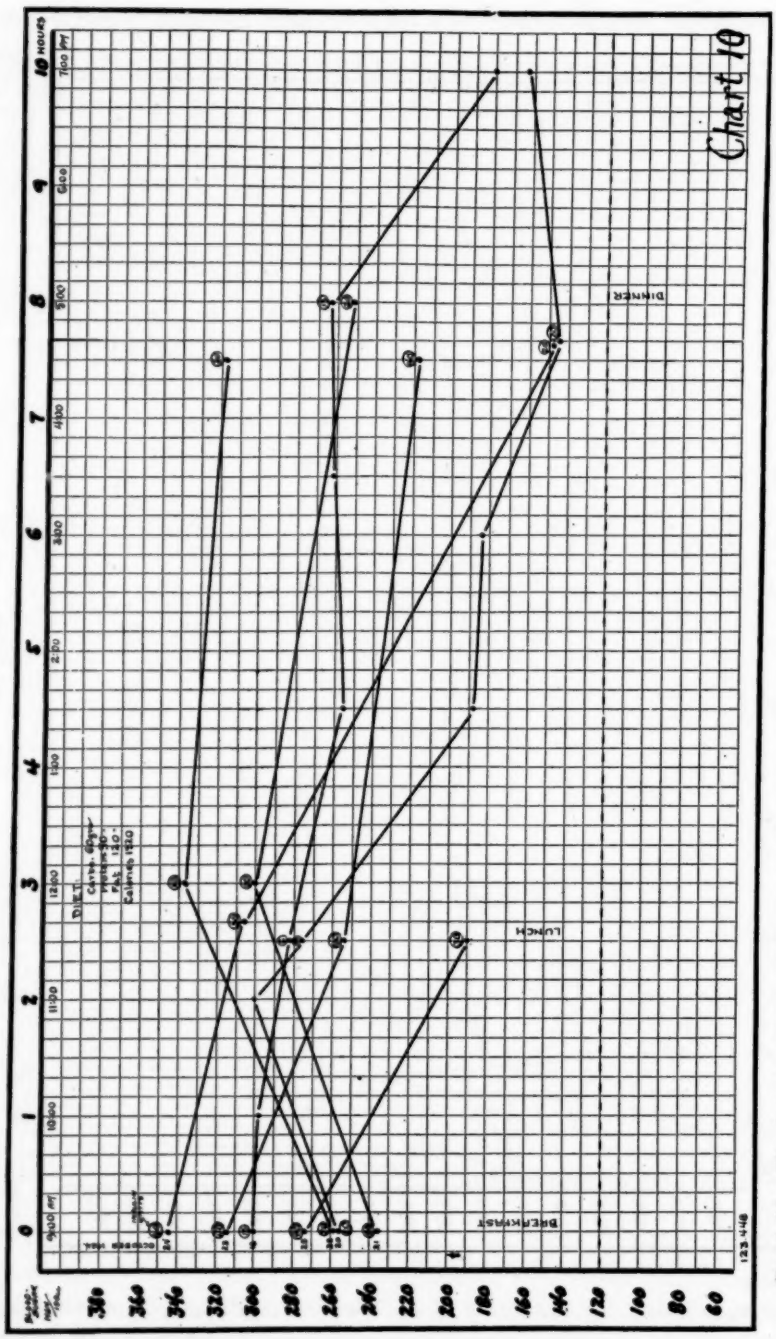

Chart 10.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on seven successive days.

The outstanding features of the action of insulin in this case were the following: (1) A fairly uniform decrease in the blood sugar from a very high level; (2) a very slight decrease in the blood sugar when it was at or slightly below the normal level; (3) no change in the blood sugar after a hypoglycemic level has been reached; (4) no reactions when blood sugar was as low as 42 mg./100 c.c.

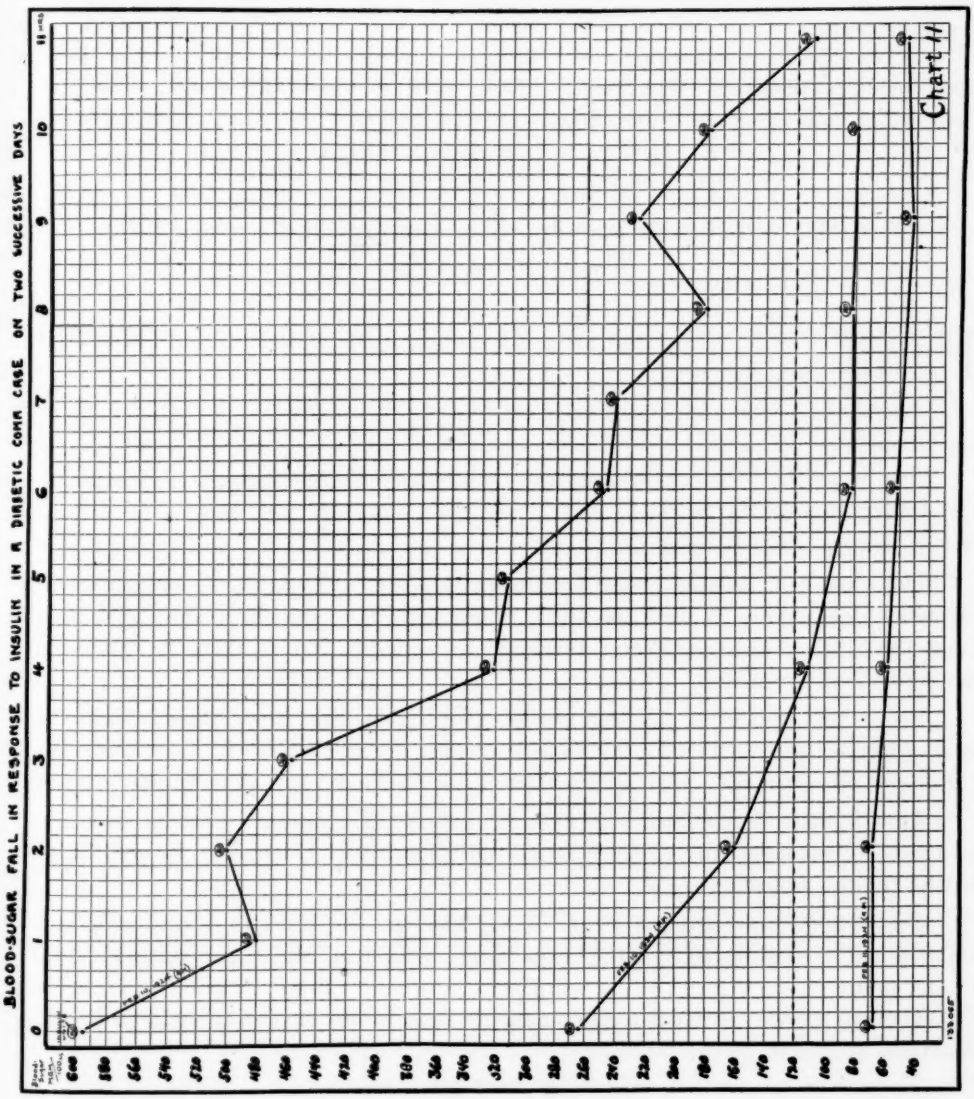

Chart 11.—Blood-sugar curves showing variations in the response to insulin in a diabetic patient on two successive days.

CONCLUSIONS

1. The amount of insulin administered intravenously appears to bear no regular relation to the fall of blood sugar.

2. While the administration of insulin usually produces a decrease in the blood sugar, in some instances it is followed by an increase which may or may not be related to postprandial hyperglycemia.

3. In general, whatever the primary effect of insulin, repeated doses result in a decrease in the blood sugar; this apparently cumulative effect, however, is independent of the size of the doses.

4. The level of blood sugar per se is not a criterion of the probability that a reaction will follow the administration of insulin. Thus a large dose of insulin may be given in the presence of hypoglycemia without any resultant reaction; and in another case a reaction may occur in the presence of a marked hyperglycemia.

A PROBLEM IN INSULIN THERAPY*

By William J. Mallory, A.M., M.D., and Joseph H. Roe, Ph.D.,
Washington, D. C.

THE general principles underlying the management of diabetes are now well established and accepted.

One of these principles is individualization. Each patient presents a peculiar problem.

Diet must be given with accuracy, according to the special requirements and limitations of the patient, and, when the necessary diet cannot be metabolized, insulin must be given.

The dose of insulin must be quantitatively adjusted to the patient's tolerance deficiency. A dose too small is bad, one too large is worse. Not only must the dose be adequate and accurate in amount, but it must be given at the physiologic moment.

From the beginning of the use of insulin, it has been known that the dose must be administered just before meals, in order that it may meet the metabolic requirements during the absorption of food from the digestive tract.

That it may be required also after the absorption period; (except in coma, and during surgical operations) is not so generally known, but is well illustrated by the following case.

Diabetes was recognized in J. W. at the age of six, two years ago. He complained of feeling tired; there was thirst, polyuria, and 6 per cent of sugar in the urine.

Dieting produced little relief, and he was taken to a hospital, where he contracted scarlet fever.

There was no gain in weight for nineteen weeks.

Insulin was then given, up to nine units twice a day, with an increase of weight from 44 to 52 pounds.

On July 24, 1924, he fell and fractured the right ulna. Two days later he was admitted to the George Washington University Hospital in diabetic coma, from which he promptly recovered under insulin treatment.

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*Read before the Medical Society of the District of Columbia, May 13, 1925.
From George Washington University.

The fracture was reduced, and, later, infected tonsils removed and abscessed teeth extracted.

From the amount of sugar in the daily twenty-four-hour urine, and the high morning blood-sugar level, it appeared that the patient required increasing doses of insulin.

The dose was, therefore, gradually raised from ten units just before breakfast, lunch, and dinner, to twenty-two units, given at the same periods.

This did not produce the anticipated result. Sugar continued to be excreted, and the morning blood-sugar level remained high, as shown in Chart I. On a day when ten grams of sugar was excreted and the morning blood sugar was 337 mg., there occurred a severe insulin reaction,—and the blood sugar

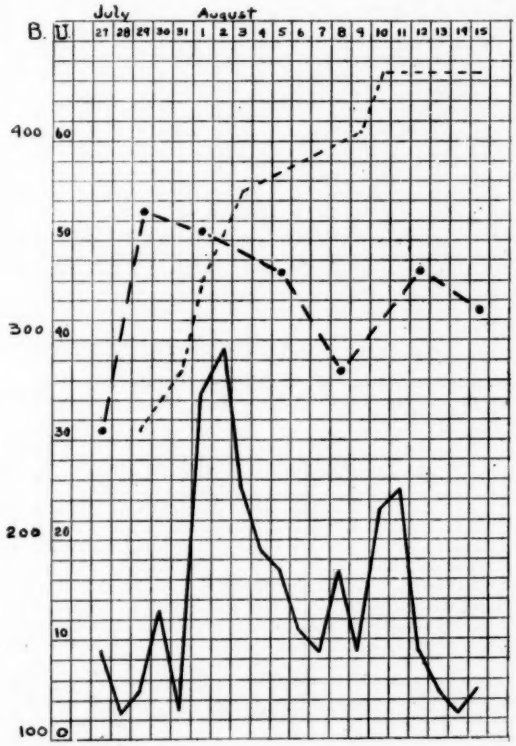

Chart 1.—Sugar excretion in grams, unbroken line, ————; blood sugar in milligrams per 100 c.c. of blood, broken line, - - - - - - - - - -; insulin in units (U.20), dotted line, ...; observe marked increase in insulin dosage without corresponding decrease in blood sugar and only slight decrease in sugar excretion.

was found to be 52 mg. per 100 c.c. The urine was then collected every three hours for the twenty-four-hour period, and the sugar determined quantitatively in each specimen. The result is shown in Chart 2.

Chart 3 shows the relation between sugar excretion, blood-sugar level, and insulin administration.

The midday dose of insulin was then omitted and 10 units given at 5 A.M. The result is shown in Chart 4.

An effort was then made to distribute the insulin dosage to meet the requirements of the three meals and also to control the hyperglycemia between 12 midnight and 7 A.M.; the result is shown in Chart No. 5.

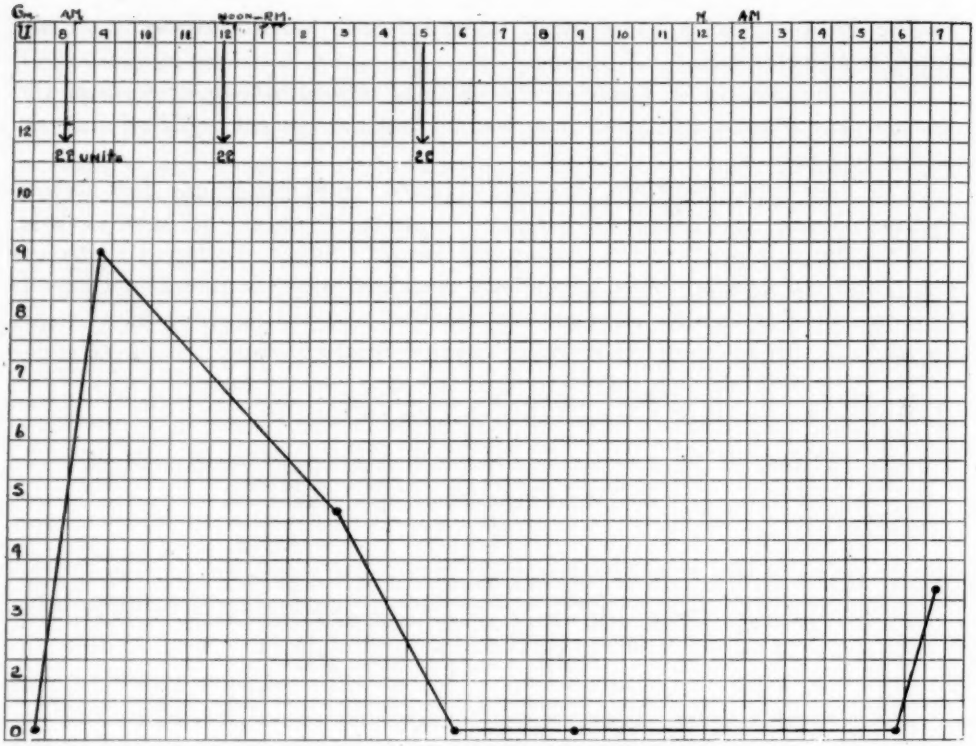

Chart 2.—Observe the excretion of 9 grams of sugar between 7 A.M. and 9 A.M. the gradual decrease to zero at 6 P.M., where it remained till 6 A.M., followed by the excretion of 3.5 grams between 6 A.M. and 7 A.M.

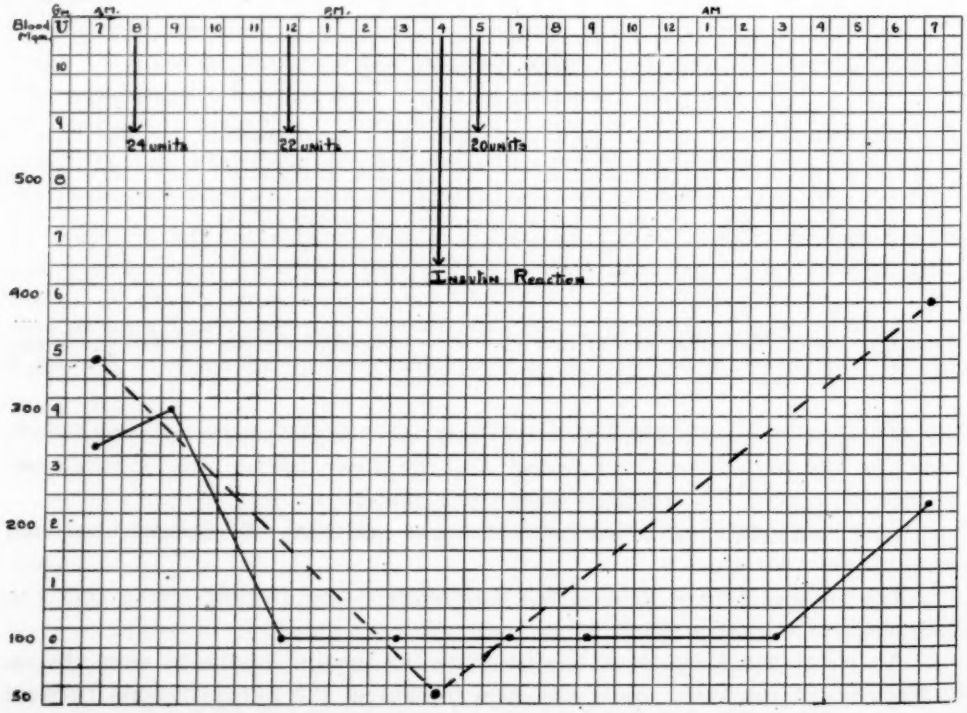

Chart 3.—Observe that while no sugar was excreted between 12 noon and 3 A.M., 2.5 grams were excreted between 3 A.M. and 7 A.M.

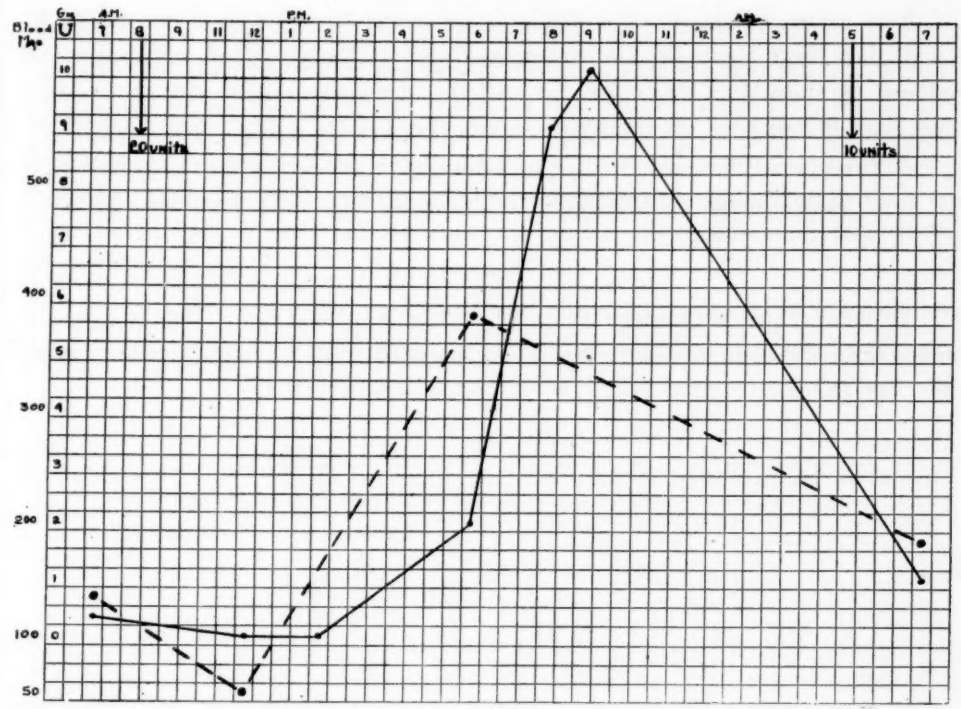

Chart 4.—Observe the effect of the omission of the insulin at noon, and the effect of 10 units at 5 A.M. on the morning blood-sugar level.

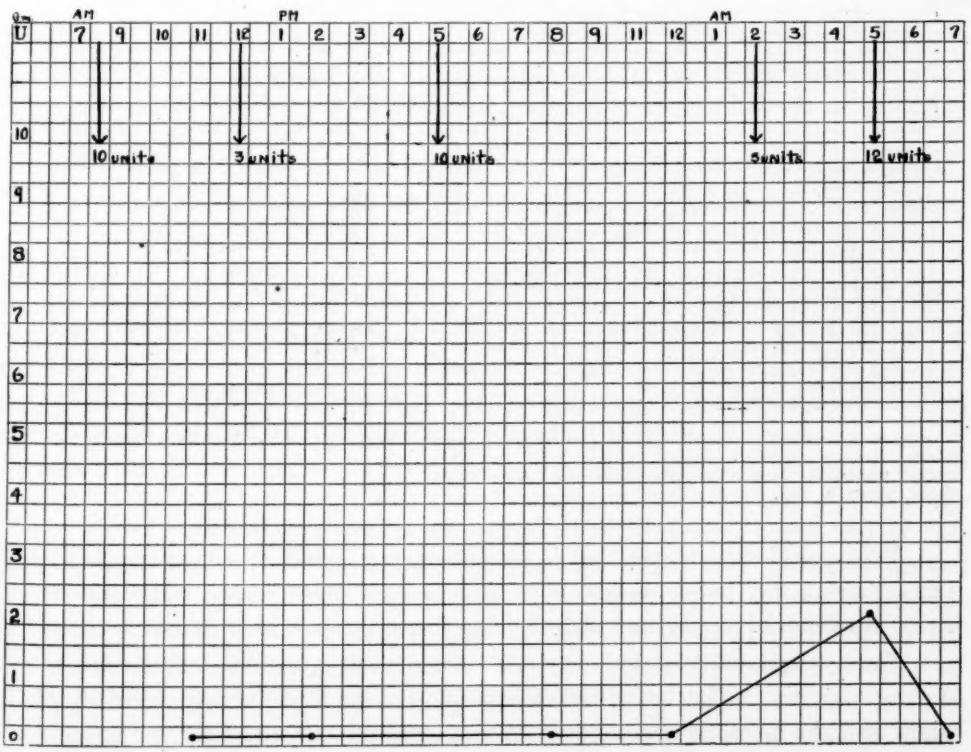

Chart 5.—After redistribution and reduction of insulin dosage to 38 units. Observe the control of sugar excretion till midnight. Two grams of sugar were still excreted between midnight and 5 A.M. Compare with Chart 1, when 64 units were given in three doses.

Two grams of sugar were still excreted some time between midnight and 5 A.M., but there was some improvement, in that the total dose of insulin was reduced by twenty-six units per day and the blood sugar kept at a much lower level than it had been on twenty-two units three times a day. See Chart 6.

A further adjustment in time of administration permitted a reduction of the total daily dosage to thirty-five units, with the result shown in Chart 7.

COMMENT

Of more than a hundred cases requiring insulin, this is the first one in which three doses of insulin a day, when matched with a properly proportioned diet, has failed to control the hyperglycemia and glycosuria.

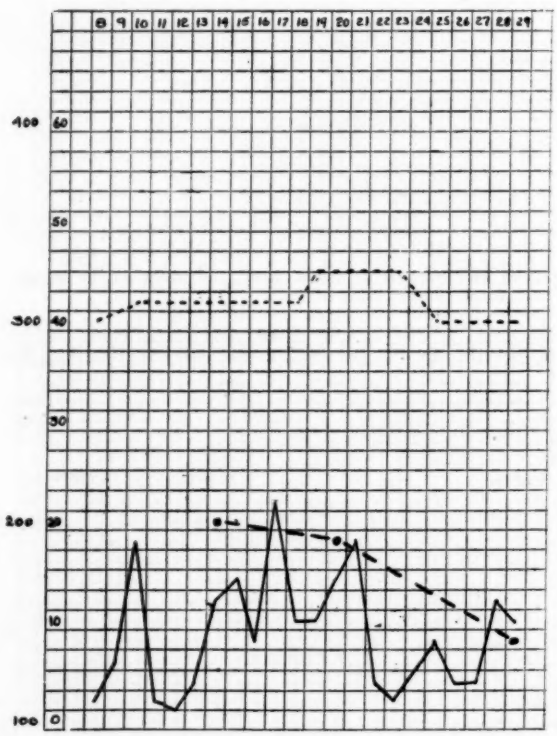

Chart 6.—Compare with Chart 1. Notice lower insulin dosage properly spaced through the day and night produces better results than a large dose three times a day, as in Chart 1.

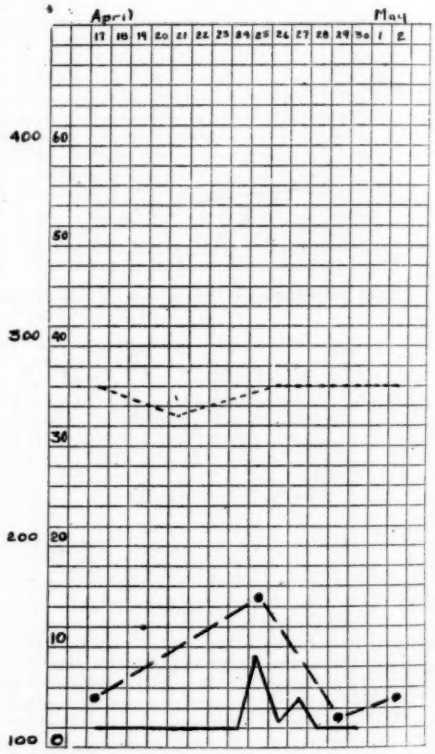

Chart 7.—Compare with Chart 1 and Chart 6.

This case demonstrates that a patient on a weighed diet, receiving insulin three times a day before meals, may show a high blood sugar in the morning, daily excretion of sugar, and still develop hypoglycemia of a dangerous degree.

The hypoglycemia is obviously due to the overlapping effect of insulin doses during the day. The hyperglycemia and glycosuria occurring between midnight and the morning dose of insulin may mislead one and lead to the dangerous increase of dosage of insulin.

CONCLUSION

In a patient apparently not responding to increasing doses of insulin by a proportionate decrease in sugar excretion and reduction of blood-sugar level, the time relation of sugar excretion to insulin dosage should be investigated. The simplest method is the examination of the three-hour portions of urine. Frequent blood-sugar determinations give additional information.

The remedy is the redistribution of insulin in amount and at the time when required.

NOTE.—Since the above was written, four additional cases have been observed which behaved in a similar manner, that is, they required the administration of insulin some time between midnight and 6 A.M. in order to maintain the blood-sugar level approximately normal.

BLOOD GAS ANALYZER*

BY C. C. GUTHRIE, M.D., PH.D., PITTSBURGH, PA.

THE importance of blood gas analysis entitles it to a place in undergraduate medical physiology and pharmacology. It is particularly instructive in conjunction with simultaneous measurement and analysis of expired air, in rebreathing experiments, and in pharmacologic studies. The available forms of such analyzers are too costly for some medical schools to purchase, or require more time for mastery by the average student than is available in such courses. For these reasons the following apparatus was devised. Its class use for three years has been so satisfactory that it seems desirable to describe the design and record data essential to its evaluation.

The apparatus is a modification of the Haldane form[1] without the control tube and supplied with a pressure equalizer consisting of a cylinder and hollow glass plunger in place of the conventional reservoir and flexible tube. So constructed, the apparatus is very compact and inexpensive (Fig. 1).

The burette (*A*) is made from a one c.c. pipette graduated in hundredths, by bending the suction end back upon itself just above the zero calibration mark and removing the constricted portion of the tip. The equalizing cylinder (*B*) is fitted with a hollow plunger (*C*) consisting of a piece of thick-walled glass tubing having an internal bore the same as that of the burette. The plunger should be fitted snugly but not tightly so that it may be easily raised and lowered. If necessary, the final fitting may be perfected by rubbing down the plunger with carborundum paper. The lower or curved end of the displacing cylinder is closely connected to the tip end of the burette by means of a short rubber sleeve (*D*). The assemblage is mounted on a wooden support (*E*) by means of two brass spring clips (*F* and *G*) attached with screws. A flat brass spring (*H*) is mounted just above the upper end of the equalizing cylinder and so adjusted that it presses moderately against the plunger to

*From the Department of Physiology and Pharmacology, School of Medicine University of Pittsburgh, Pa.

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insure that the plunger will remain stationary in all positions. The wooden base measures 11 by 2¼ by ½ inch. In the middle of the back, three inches from the top, a rod ¼ by 1½ inches, threaded for ½ inch is screwed into a hole provided for the purpose and serves as a support for clamping the apparatus to a stand (*I*). The wooden base is painted black and a strip of white glazed paper pasted to the front before mounting the burette to serve as a background for accurate reading. The dimensions of the glass parts are such

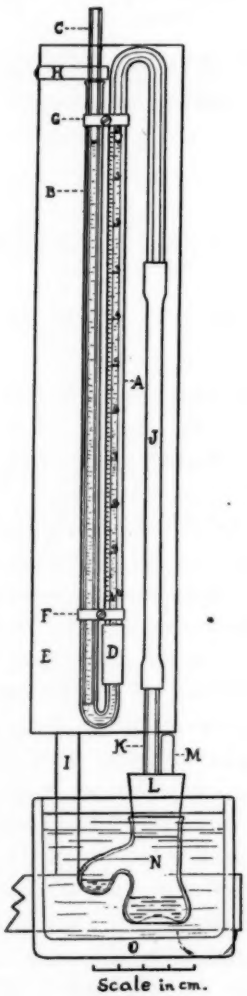

Fig. 1.

that when mounted the tubes do not project beyond the margins of the base, excepting the upper end of the plunger which should be of such length that when thrust to the bottom of the cylinder the upper end projects ½ inch above the base. The free end of the gas burette is connected by means of a seven-inch length of thick-walled pure gum tubing (*J*) to a straight glass tube (*K*) having a bore of 4 mm. The glass tube is inserted into a hole of a two-holed No. 4 rubber stopper (*L*) until the end is flush with the under surface. To close the other hole in the stopper a piece of glass rod (*M*) about one inch

long is employed. A glass vial (*N*) provided with a side pocket is connected with the stopper. The neck of the vial is of such size that a secure fit is obtained when the stopper is inserted ¼ of an inch. The total height of the flask is 1⅝ inches. The bottom is flattened and flared. The neck of the side pocket arises near the middle of the wall. The neck should not be less than ⅜ of an inch in diameter and the pocket is depressed and flared so that it holds at least 0.5 c.c. without spilling over into the vial when the vial is placed upright on the table. The total capacity of the vial with cork in position is 13 c.c. The base is clamped to the stand and an 8 oz. ointment jar (*O*) of water is set beneath and the apparatus lowered until the vial is suspended in the water so that the upper surface of the water comes to the level of the junction of the rubber stopper and vial mouth.

Alcohol, or water to which a little bile salts have been added,[2] is used for the displacing liquid. Before connecting the vial, or before sealing the vial with the glass plug, with the plunger pushed down as far as it will go the alcohol level should be at slightly above zero on the burette.

Accessory apparatus comprises a thermometer, pipettes calibrated for measuring 0.5 and 1 c.c. of blood and an aerating bottle, which will be described with the directions for its use.

The reagents are (1) ammonia solution. This consists of one part of strong ammonia (sp. gr. 0.88) made up to 250 parts with boiled distilled water.[3] To this a small quantity of saponin powder is added to accelerate laking of the red blood corpuscles and several drops of caprylic alcohol to prevent foaming on shaking after adding the blood. This is conveniently kept in a bottle provided with a pipette graduated to measure 1.5 c.c. (2) A saturated solution of potassium ferricyanide. (3) A 20 per cent solution of tartaric acid. Both the ferricyanide and tartaric acid solutions are kept in small bottles provided with dropping pipettes. (4) Saponin powder.

APPARATUS ASSEMBLAGE AND ADJUSTMENT

Push the displacing plunger well down. The column of alcohol in the tubes should read near, or slightly above zero. Remove the glass plug and connect the rubber stopper to the tube so that when it hangs down, the hole in the stopper for the glass plug is directed forward. Attach the vial to the stopper firmly. Place the water-bath in front of and against the stand and beneath the vial so that the vial hangs near the middle of the bath. By raising and lowering the apparatus on the stand, make adjustment so that the vial is submerged to the top of the lip. Note the room and bath temperatures. The temperature of the bath should not be more than about 0.5° C. below the temperature of the room. Do not work in a draught, in direct sunlight, or near a radiator.

CONTROL TEST

Place two drops of the ferricyanide solution in the vial pocket and 1.5 c.c. of the ammonia solution in the vial. Insert the stopper and place the vial in the water-bath, taking care not to mix the reagents. Agitate the water in the bath for five seconds by holding to the stopper and giving the vial a gentle

circular motion. Moisten one end of the glass plug and insert it about ¼ inch in the hole in the stopper. This will depress the alcohol in the gas burette. Equalize the levels of the two columns of alcohol by raising the displacing plunger. At about one minute intervals, stir the water-bath with the vial by grasping the stopper only, as previously described. As may be necessary, readjust the levels of the alcohol columns until they show no change for a period of one minute. This will indicate the zero reading. The burette may be read to 0.1 of a space, i.e., 0.001 c.c. In general, the zero reading should be adjusted to slightly below the zero mark. When necessary to readjust for this, it is easily accomplished by removing the glass plug and readjusting the alcohol levels to slightly above zero before reinserting the plug. After zero is established and recorded, by grasping the stopper, lift the vial from the bath and thoroughly mix the contents of the vial and pocket by tipping and gently shaking from side to side for two minutes. Do not permit any of the liquid to get on the stopper. Replace the vial in the bath and observe any change in the volume of gas by the technic above described for establishing zero level, excepting that the glass plug is not touched. When the volume has become stabilized, note any change. Any volume change is obtained by subtracting the zero from the final reading.

Remove the glass plug, unstopper the vial and wash, rinse with distilled water at room temperature and dry it.

OXYGEN CAPACITY OF BLOOD

Blood is prepared by placing about 5 c.c. in a clean, dry, six-ounce, wide-mouth bottle, replacing the stopper and rotating horizontally for five minutes. Shaking or agitating with sufficient violence to cause foaming is avoided.

The procedures described for the control test are now repeated and in addition, 1 c.c. of the oxygenated blood is placed under the ammonia solution. The surplus blood from the point of the pipette is removed with a pledget of cotton before putting it under the ammonia solution. Shake gently to mix the blood and ammonia solutions and wait at least five minutes for the zero reading in order *to insure that the blood will have become completely laked before mixing with the ferricyanide solution.* In mixing the ferricyanide with the blood and ammonia and shaking, avoid the formation of bubbles as they interfere with the accuracy of the results. As the gas is given off keep the alcohol levels approximately the same by raising the displacing plunger. The final reading should be obtained in from four to five minutes. The end-point, of course, is indicated by the volume becoming stable. Note the bath temperature. The final result is expressed in cubic centimeters of oxygen per 100 c.c. of blood. Reduce this to dry gas at 0° C. and 760 mm. Hg. pressure. Table I is adequate for student use.

OXYGEN CONTENT OF ARTERIAL BLOOD

This is determined as in the case of oxygen capacity, excepting that the blood is not exposed to air but removed from beneath oil and placed in the vial under the ammonia. (Caution.—On placing the blood under the ammonia solution do not agitate until the zero reading has been established.) Set zero at about 0.10, then shake gently to lake and continue until the blood is saturated

TABLE I

FACTORS FOR REDUCING TO DRY AIR AT 0° C. AND 760 MM. OF AIR SATURATED WITH MOISTURE AT DIFFERENT TEMPERATURES AND PRESSURES. PRESSURE 735 MM. HG.

15° C.	16°	17°	18°	19°	20°	21°	22°	23°	24°	25°
0.898	0.894	0.889	0.884	0.880	0.875	0.870	0.865	0.860	0.856	0.850

with oxygen. Read the burette to determine the amount of oxygen absorbed.* Then mix with the ferricyanide and determine the total amount of oxygen.

The second reading subtracted from the first reading gives the amount of oxygen absorbed in the vial; while the first reading from the last reading gives oxygen content of the blood when placed in the vial. The second reading from the last reading gives the oxygen capacity but the method first described is recommended.

OXYGEN CONTENT OF VENOUS BLOOD

The technic is the same as for the oxygen content of arterial blood. From the oxygen capacities of the arterial and venous blood samples the oxygen contents can also be expressed in percentages of saturation.

CARBON DIOXIDE CAPACITY OF BLOOD

Direct Method.—This is determined with the same apparatus as used for oxygen capacity and the general technic is the same. In addition to carbonate-free ammonia solution and saturated ferricyanide solution, 20 per cent tartaric acid solution is required. It is most important, if carbonate is present in the ammonia solution, that the quantity be known so that the figure for blood can be corrected. To determine this, place 4 drops of tartaric acid in the vial pocket, 1.5 c.c. of ammonia solution in the vial and proceed as described in the control experiment under oxygen capacity.

To determine the carbon dioxide capacity of blood, place 5 c.c. in a six-ounce, wide-mouth bottle provided with a tight stopper through which two glass tubes extend into the bottle. The tubes are closed by small stoppers or caps. After the blood has been placed in the bottle, add a little dry saponin powder and agitate occasionally until the blood is *completely* laked. This should occur in about five minutes. Then add two drops of saturated ferricyanide solution and shake gently until the oxyhemoglobin has been changed into methemoglobin. This requires not more than five minutes. It can be determined spectroscopically without removing the blood from the bottle. If oxyhemoglobin is present, on subsequent addition of tartaric acid and shaking, some of it will be liberated, thus interfering with the accurate determination of carbon dioxide.

The blood is now exposed to alveolar air until the carbon dioxide content becomes stabilized. To do this, unstopper the tubes and at the end of expiration forcibly expel all the air possible from the lungs into the bottle. Do this two or three times to insure that all of the air originally in the bottle has been displaced by expired air. Then quickly stopper the tubes, place the bottle horizontally on the table top and roll it to and fro so that the blood forms a film around the sides of the bottle. After two or three minutes, again

*For observing the rate of absorption as well as the total amount of oxygen absorbed, a method including a description of a constant shaking apparatus has been described by McElroy and Guthrie, Am. Jour. Physiol., 1920, li, 195.

expire into the bottle as before, close the tubes and continue to roll the bottle for several minutes. If the bottle temperature is below the dew-point, some moisture from the breath will condense. In such case, the expired air may be introduced through a bottle of glass beads or other device in order to remove the surplus moisture. Ordinarily, this is unnecessary.

Place four drops of tartaric acid in the pocket of the vial and 1.5 c.c. of the ammonia reagent in the vial and with the pipette place 0.5 c.c. of blood under the ammonia solution. In doing this, avoid exposure of the blood to room air. The vial is stoppered and a test for carbon dioxide carried out as before described.

The actual volume percentage of carbon dioxide is calculated from the burette reading as follows:

1. Correct the reading by subtracting the amount of carbon dioxide given off by the ammonia solution alone on mixing with the acid as determined in the control test.

2. Add 2.0 per cent of this volume to correct for dilution of blood by ferricyanide in aerating bottle and double this to express the amount of carbon dioxide in 1 c.c. of blood.

3. To correct the reading for the amount of carbon dioxide remaining dissolved in the solution, multiply the number of degrees the temperature of the bath is above 13° C. by 0.5, subtract this from 120 and multiply the corrected gas reading by the remainder.[4]

4. By reference to the table given under oxygen capacity, correct the gas volume for water vapor, temperature, and pressure.

CARBON DIOXIDE CAPACITY OF BLOOD

Indirect Method.—To determine the carbon dioxide capacity of blood by the indirect method proceed as in the direct method, placing five c.c. of blood in the aerating bottle, adding saponin powder to lake.

A. After laking the blood, saturate it as previously described by exposure to alveolar air. Remove 1 c.c. and determine the oxygen content as previously described.

B. Again expose the blood in the bottle to alveolar air and place 0.5 c.c. under the ammonia solution in the testing flask, into the pocket of which two drops of ferricyanide solution and four drops of tartaric acid solution have been placed and determine the total oxygen and carbon dioxide content.

The actual volume percentage of carbon dioxide is calculated from the burette reading as follows:

1. From the total volume, subtract the amount of carbon dioxide given off by the ammonia solution alone, on mixing the acid and one-half of the oxygen content of 1 c.c. of blood as determined in the previous tests.

2. Correct for the amount of carbon dioxide remaining dissolved in the solution as described under the direct method. A more exact correction would be the addition of 21.5 per cent less a fortieth for each degree above 13° C., but the difference in the final result would be of little significance.

3. Double this to express the amount of carbon dioxide in 1 c.c. of blood.

4. Correct the gas volume for water vapor, temperature, and pressure.

OXYGEN AND CARBON DIOXIDE CONTENT OF ARTERIAL AND VENOUS BLOOD

The blood is collected under oil and analyzed for oxygen alone, and for total oxygen and carbon dioxide as described under the indirect method for carbon dioxide capacity under A. and B., excepting that the blood, as in the case of the oxygen determination alone, is transferred from under the oil to under the ammonia solution.

By determining the oxygen capacity of the blood by saturating in alveolar air the oxygen contents of arterial and venous blood in terms of percentage saturation may be expressed.

DISTRIBUTION OF OXYGEN AND CARBON DIOXIDE IN BLOOD

Divide a sample of oxalated blood. Centrifugalize one sample to obtain plasma. Determine the oxygen and carbon dioxide capacity of each. By determination of the volumes of the sediment and of the plasma, the actual quantitative distribution in the whole blood may be calculated.

SUMMARY

The methods have yielded sufficiently accurate results for the purposes for which they have been employed. Check tests are always made, and in reasonably practiced hands, usually vary not more than 5 per cent. Results for oxygen are more uniform than for carbon dioxide. Oxygen capacity tests are the most accurate and uniform while carbon dioxide content tests are least uniform. As with all gas methods, increasing skill is shown by greater uniformity of results, and repeated tests have demonstrated that in only moderately trained hands, results by these methods certainly are as accurate and uniform as by other current methods, while the time necessary to learn the technic as well as the time required for making tests is shorter.

REFERENCES

[1]Haldane, J. S.: Respiration, Yale University Press, 1922, p. 410.
[2]Haldane, J. S.: loc. cit., p. 412.
[3]Haldane, J. S.: loc. cit., p. 416.
[4]Haldane, J. S.: loc. cit., p. 416.

BASIC FUCHSIN FOR ENDO MEDIA*

By R. W. French, Washington, D. C.

PRELIMINARY consideration of the action of the basic fuchsin-sodium sulphite combination in an Endo medium discloses the fact that the reaction is practically independent of changes in hydrogen-ion concentration, but in reality that the combination is acting largely as a Schiff's reagent, detecting aldehydes produced by bacterial metabolism. This statement may be readily proved experimentally, whereby it is found that sensible variations in P_H utterly fail to influence the appearance of color in such a medium.

*From the Laboratory Service, Walter Reed Medical Center, Washington, D. C.
Received for publication, June 5, 1925.

With this fact established the following test for the suitability of a basic fuchsin for use in Endo media is suggested:

In a test tube take 10 c.c. of a 0.05 of 1 per cent aqueous basic fuchsin. Determine the amount of 0.5 of 1 per cent aqueous sodium sulphite necessary to effect decolorization. Then determine the amount of 0.5 of 1 per cent formalin (40 per cent formaldehyde) necessary to restore a distinct fuchsin color (the original color will not be restored).

In use, this test first will show that samples of fuchsin vary considerably in performance, some requiring very little of the sulphite to effect complete decolorization, likewise little of the aldehyde to restore the color. Other samples will require many times the amount of sulphite and formaldehyde for appreciable results.

The application of this test on the different types of basic fuchsin reveals some interesting facts. First, in the case of a new fuchsin or a para-rosaniline hydrochloride a very appreciable precipitate forms immediately on the addition of the sodium sulphite, while with rosaniline hydrochloride no precipitate is noticeable for some time. Second, the addition of but a few drops of the formaldehyde solution restores the color to the decolorized rosaniline hydrochloride, while many times the amount of formalin is necessary to effect a partial restoration of color to the new fuchsin. Third, the precipitate formed on the addition of the sodium sulphite persists even after adding the formalin. Filtration further shows that the filtrate from the para-rosaniline hydrochloride is colorless (that the formalin has failed to restore any trace of color) while the filtrate from the new fuchsin shows but a very slight restoration of the original color.

These results, which parallel quite well those obtained in the investigations by the Biological Stain Commission,* show that rosaniline hydrochloride is thoroughly satisfactory for the preparation of an Endo medium, that new fuchsin is partially so, and that para-rosaniline hydrochloride is entirely unsatisfactory.

From the degree of the reaction in the test tube it is apparent that a small portion of the new fuchsin molecule acts similarly with sodium sulphite to rosaniline hydrochloride, which is not surprising when it is considered that chemically the two compounds are similar, the former having two more methyl groups in meta position to the methane carbon. It also seems, judging from the great difference exhibited by para-rosaniline hydrochloride, that the methyl group in meta position to the methane carbon in rosaniline hydrochloride is an essential to the developing of a color by the aldehyde after decolorization of a fuchsin by sodium sulphite.

Quantitatively, rosaniline hydrochloride is much the more satisfactory for Endo media than any other of the basic fuchsins, or mixtures thereof. The color is developed quicker and to a much greater degree than is the case with samples of new fuchsin ordinarily, and where this latter dye works satisfactorily or on a parity with the rosaniline, it is undoubtedly due to an admixture of the latter dye.

NOTE: Since the preparation of this article reference by Muller, compt. rend. soc. biol., 1924, xciv, 653-655, is noted in which it is shown that an acid also is necessary to accomplish the reaction. Any organic acid, such as lactic acid, may be used, and as a rule there is sufficient formic acid in the formaldehyde to bring about the reaction.

*Science, lx, No. 1556, pp. 387-388.

LABORATORY METHODS

A NEW HEMOGLOBIN SCALE*

By C. E. Roderick, M.D., Battle Creek, Mich.

THE small instrument shown in Fig. 1 is used for the estimation of hemoglobin. It is especially designed for the use of the practicing physician at the bedside.

It is not claimed that this instrument will replace the Dare, Fleischl-Meischer and others of the more expensive type which are to be found in the Clinical Laboratory.

It consists of a color scale graduated from 10 to 100 per cent. One hundred per cent being equivalent in color to a standard base on 13.77 gm. of hemoglobin in 100 c.c. of serum. This is the standard used by other instruments of present-day use.

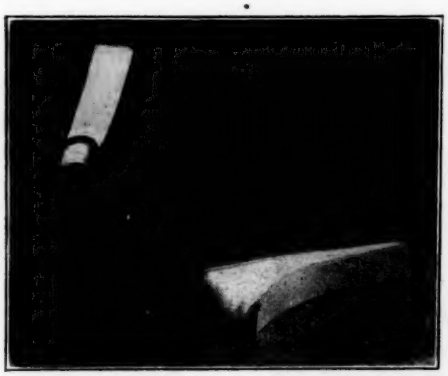

Fig. 1.

While no paper color scale can be relied upon to determine low percentages of hemoglobin with complete accuracy, this scale agreed with the Dare instrument in a large number of cases where the hemoglobin was found to be forty per cent or more, and when there was disagreement, it was seldom more than eight per cent.

The technic for use of this instrument is simple:

A small leaf of paper is removed from the pad (Fig. 2-*A*) and a drop of blood is soaked up. This is then placed in the small frame above the aperture and the edge brought into apposition with the color scale inside (Fig. 3). The scale is then turned by means of the little thumb-screw and the color is matched and reading made directly at the base of aperture.

*From Battle Creek Sanitarium.

Read before the Fourth Annual Convention of the American Society of Clinical Pathologists at Philadelphia, May 20-23, 1925.

The advantages of the instrument are:

1. Small and compact; easily carried in the vest pocket, Fig. 4.
2. Simple to operate.
3. Economical; uses small pieces of paper which are made up in booklets.
4. Efficient in that the areas of blood and scale compared are about 1 cm. square.

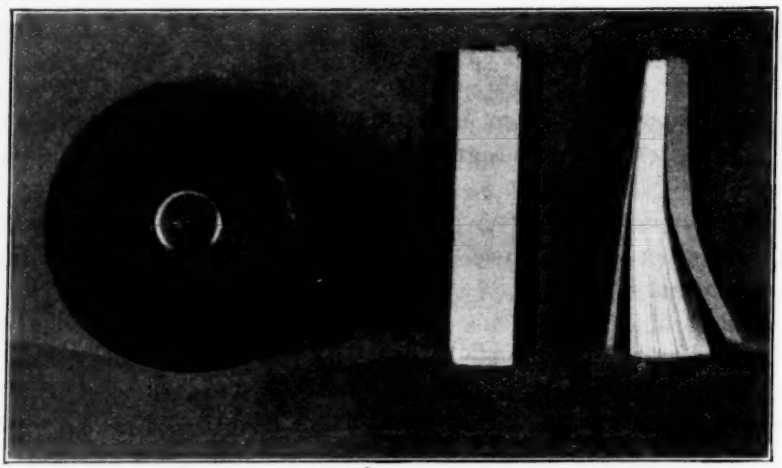

Fig. 2. A.

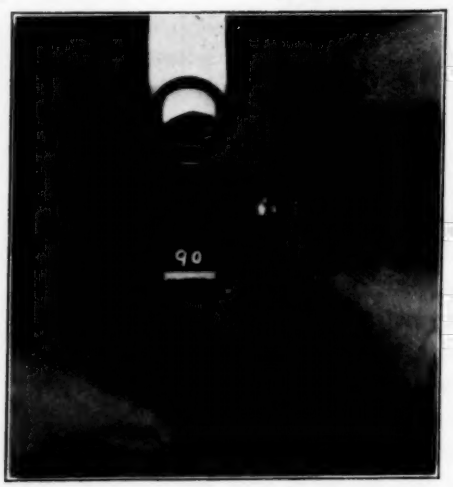

Fig. 3.

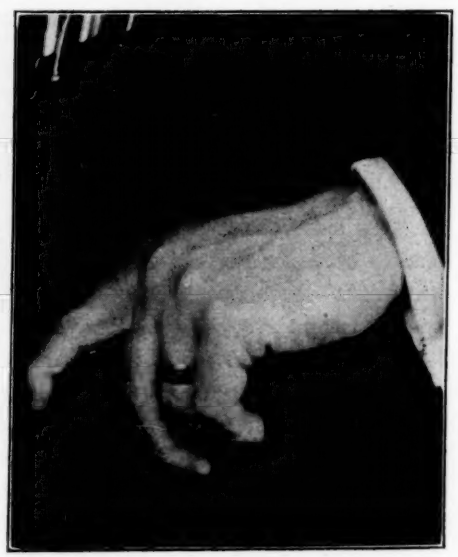

Fig. 4.

5. Scale is covered, with exception of one shade, when not in use, thus fading is prevented.
6. Made of metal and will wear for years. Additional booklets of paper may be had, thus eliminating the necessity of purchasing a new scale each time the paper supply is exhausted.
7. Should paper scale fade or become otherwise damaged it may be replaced without the necessity of securing another instrument.

ANILIN AS A DECOLORIZING AGENT IN THE GRAM STAIN*

By Hardy A. Kemp and Moyer S. Fleisher, St. Louis, Mo.

A SCORE or more of modifications of the Gram technic for staining and differentiating bacteria have been offered since Gram's original work in 1884. Most of these modifications have for their purpose the elimination of those variable factors which render the results obtained with this method more or less uncertain in the hands of the inexperienced, and even at times in the hands of more experienced workers. Using the original Gram technic, the principal cause of variations in results seemed to center on the decolorizing agent, namely, alcohol.

In an effort to find a method which would give constant results, even when used by the beginner in bacteriologic technic, we were led to try various modifications in which a decolorizing agent other than alcohol was used. We used successively acetone, acetone-alcohol, acetone-phenol, acetone-ether, phenol, and alcohol in percentages ranging from twenty-five to absolute. In these, as in all later experiments except when expressly stated, Staphylococcus aureus was used as the representative Gram-positive organism, and Friedländer's bacillus as the Gram-negative organism. The results with all of these reagents were, however, indifferent at best, and apparently uncontrollable factors, or factors very difficult to control, entered into the differentiating by means of all of these agents. Even when a rigid technic was used, they did not give constant results with the two test organisms.

There is mention made in Mallory and Wright's *Pathological Technique*,[1] of Weigert's method of staining bacteria in tissues. Weigert's technic[2] is essentially similar to the Gram method for staining bacterial smears, excepting that anilin is used as a decolorizer and no counterstain is employed. We have found no other references in the literature concerning the use of anilin as the decolorizing agent in the Gram stain. The results which we have obtained with anilin as a decolorizing agent have, however, been strikingly constant.

We found on decolorizing with anilin after staining according to Gram's method, using however ammonium oxalate gentian-violet[3] in the first step, that the counterstain in every instance brought out the Gram-negative bacillus in unmistakable contrast to the Gram-positive coccus. In all cases in which the smear had been thoroughly washed in the anilin, no bacillus could be found which retained the violet color, nor were there any red staining cocci. Subsequent trials with other organisms gave similar results.

The technic employed is as follows:

1. Ammonium oxalate gentian-violet applied to the smear for one minute.
2. Wash off excess stain in tap water.

*From Department of Bacteriology and Hygiene, St. Louis University School of Medicine.
Received for publication, September 26, 1925.

3. Gram's iodine—at least two minutes. (There has been some question as to whether Gram's iodine is any more effective after a period of time longer than one-half minute. Our best results were unquestionably obtained with a two-minute period.)

4. Wash off excess iodine with tap water and dry smear thoroughly.

5. Wash the smear in a bath of anilin (preferably C.P.) for at least a minute. Wash until there is no blue color in the film of oil that runs off the slide when the slide is lifted from the bath for inspection. The first dip will bring the purple color away in a flood. This is not sufficient. The slide must be washed in the oil until there is no longer any trace of the dye in the washings. We have found that prolonged and vigorous washing will not remove the violet from Gram-positive organisms but that insufficient dippings will fail to decolorize the Gram-negative bacteria.

6. Blot off all the oil, and dry carefully by finally passing through the flame.

7. Counterstain with dilute fuchsin or safranin.

In Table I we show the influence of the length of time of decolorization with anilin upon the Gram-negative and Gram-positive organisms. This tabulation is simply a condensation of many tests carried out with the different time periods and the different organisms. It is evident that decolorization must be continued for at least one minute and that longer periods of exposure do not lead to a decolorization of the Gram-positive organisms. It is also evident from this table that this method is generally serviceable in differentiating Gram-negative and Gram-positive organisms.

TABLE I

TABULATION OF RESULTS. TECHNIC AS DESCRIBED. ANILIN AS DECOLORIZER

ORGANISM	ONE IMMERSION* OF SMEAR	TWO IMMERSIONS	HALF-MINUTE VIGOROUS WASHING	ONE MINUTE VIGOROUS WASHING	FIVE MINUTES VIGOROUS WASHING	FIFTEEN MINUTES SAME	ONE HOUR SOAKING SMEAR
Staph. aureus	G+	G+	G+	G+	G+	G+	G+
B. subtilis	G+	G+	G+	G+	G+	G+	G+
B. Friedländer's	G+G−	variable	G−>G+	G−	G−	G−	G−
B. coli	G+G−	variable	G−	G−	G−	G−	G−
B. dysenteriae Flexner	G+	variable	G−	G−	G−	G−	G−
B. dysenteriae Shiga	G+	G−G+	G−	G−	G−	G−	G−
B. botulinus	G+	G+	G+	G+	G+	G+	G+
B. anthracis	G+	G+	G+	G+	G+	G+	G+
B. tetanus	G+	G+	G+	G+	G+	G+	G+

*By immersion we mean dipping with immediate withdrawal and drying. G + = Gram-positive; — = Gram-negative.

The possible sources of error in this technic which we here suggest are relatively few: lack of thoroughness in washing in the anilin; too brief fixation time with the iodine; and possibly changes in the P_H of the stains.[4] This method does not have the merit of being a rapid method, but speed was not what we sought. We believe, however, that the technic is relatively simple and gives constant results, since decolorization can practically never be carried too far, and thus an important source of confusion in the Gram stain is removed. A number of our students instructed in this method of staining, used it in their laboratory work. The results were constant and satisfactory. With

alcohol and decolorizers other than anilin they were always at a loss to recognize that proper point of decolorization where all the Gram-negative organisms have been relieved of the violet and the Gram-positive combinations stand unattacked and unbroken. With anilin this personal factor was largely removed and the technic made less fallible.

Because the anilin can be used for some time without appreciable change in its value as a decolorizing agent, we find it as inexpensive in the long run as alcohol or any of the other agents.

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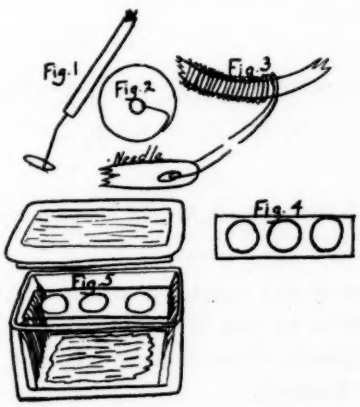
[3]Hucker, G. J.: Abst. Bacteriol., 1922, vi, 2.
[4]Stearn, E. W., and Stearn, A. E.: Jour. of Bacteriol., 1924, ix, 463-479; x, 13.

PARAFFIN RINGS ON MICROSCOPE SLIDES*

By Glenn Green, B.A., Cambridge, Ohio

USUALLY in microscopic agglutinations the practice has been to use a grease pencil in making the rings on the glass slides. The tool herewith described saves time in making the rings, they adhere firmly to the glass, prevent intermingling and loss of specimens, and are uniform in size. Paraffin ring slides are used at Lakeside and Mt. Sinai Hospitals, Cleveland, Ohio, and



are completely satisfactory in matching blood samples in transfusion cases, as well as in all sorts of bacterial agglutinations. The method was developed at Lakeside Hospital Pathological Laboratory two years ago.

The tool is a heavy nichrome culture needle set in an aluminum handle. The free end of the nichrome wire is carefully bent so as to form a circle about 16 mm. in diameter, the plane of the circle being perpendicular to the handle.

*From Cambridge Laboratory, Cambridge, Ohio.
Received for publication, September 19, 1925.

Fig. 1 is an oblique view of the tool, and Fig. 2 gives the detail of the ring. The ring must now be wrapped with thread in order to obtain even distribution of the paraffin. Cotton thread will operate as a wick and No. 20 has been found a satisfactory size to use. Fig. 3 is a detail, several times enlarged, which illustrates the method of wrapping.

In use the tool is dipped into ordinary paraffin heated to the point where it begins to smoke. It is then placed lightly but securely on a dry slide, withdrawn quickly, and the paraffin allowed to cool. Three rings can be placed on an ordinary three inch slide (Fig. 4), and by practice rapidity of technic is easily developed.

Rings of 16 mm. diameter will hold two large drops easily and allow for manipulation of the slide to effect mixing. Stirring with a flat wooden toothpick, however, will produce a much more intimate intermingling of the drops. Of course, a separate toothpick is used for each ring and then discarded. After a ten to twenty-minute incubation the whole slide may be tilted and manipulated to produce enough agitation to break up any pseudoagglutination. Cover slips cannot be used with paraffin rings. In order to prevent evaporation of the specimens during the incubation period, it is necessary to place them in a covered humidor. At the above-named hospitals the humidors used are ordinary staining dishes with wet blotting paper on the bottoms. These are illustrated in Fig. 5.

If it is necessary or desirable to use the slides again, they may be cleaned satisfactorily by boiling vigorously in a washing soda or soap powder solution and then allowing them to cool. The soap or soda dissolves dried sera or other substances, while the heat and boiling action dislodge the paraffin. When cold, the scum of solid paraffin can be removed from the top and the slides rinsed in clear water.

A NOTE UPON THE DESTRUCTION BY HEATING OF COMPLEMENT IN HUMAN SERUM*

By Robert A. Kilduffe, A.M., M.D., Atlantic City, N. J.

As a result of numerous and extended studies of the various factors influencing the application of the complement-fixation test to the serologic study of syphilis, in a majority of methods in common use, the test is conducted upon sera inactivated by heating.

The primary purpose of this inactivation is the destruction of the natural complement and of thermolabile anticomplementary substances.

Various workers have demonstrated, however, the thermolability of syphilitic reagin, and, to minimize this destruction, the period of inactivation has been reduced. Kolmer[1] recommends fifteen minutes as sufficient, and this period is rather widely used.

Davis,[2] in view of the fact that there may still be occasional doubt as to

*Received for publication, October 10, 1925.

the efficacy of this shorter period, reported the complete destruction of complement in five hundred sera inactivated for fifteen minutes at 56° C.

The present note is in further corroboration of the efficiency of this period at this temperature.

During a study of the relation of the natural hemolysins of human sera to Kolmer's quantitative complement-fixation test for syphilis, the results of which have been reported elsewhere,[3, 4] two hundred sera were subjected to a temperature of 56° C. for varying periods up to fifteen minutes and then tested for the presence of natural complement.

All of the sera tested contained in 0.1 c.c. sufficient natural antisheep amboceptor to completely hemolyze 0.5 c.c. of 2 per cent sheep cell suspension.

The sera were placed in test tubes in a water-bath at 56° C. and 0.1 c.c. removed after five, seven, ten, and fifteen minutes, placed in fresh tubes, and 0.5 c.c. of 2 per cent sheep cell suspension added and the mixture incubated in a water-bath at 38° C. for one hour.

The presence or absence of complement was determined by the occurrence or absence of hemolysis.

The results of the series were as follows:

After inactivation at 56° C. for five minutes hemolysis occurred in eight sera, or 4 per cent.

After inactivation at 56° C. for seven minutes no hemolysis occurred in any of the sera, and the same was true of the sera tested after ten and fifteen minutes' exposure to this temperature.

Exposure to a temperature of 56° C. for fifteen minutes is, therefore, an efficient procedure for the destruction of the natural complement of human sera.

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[3]Kilduffe, R. A.: The Relation of the Natural Antisheep Hemoysins of Human Sera to Kolmer's Quantitative Complement-Fixation Test in Syphilis, Arch. Dermat. and Syph., December, 1924, x, 734.

[4]Kilduffe, R. A.: The Influence of the Natural Hemolysins of Human Sera upon the Production of Anomalous Reactions in the First Tube of Kolmer's Quantitative Complement-Fixation Test for Syphilis, Jour. Lab. and Clin. Med., November, 1924, x, 2, 93.

A GASSING CHAMBER FOR SHORT EXPOSURES*

By D. C. Walton and C. A. Jones, Edgewood, Md.

IN testing the effect of gases—industrial or war—upon animals one of the first requisites is a suitable gassing chamber. The design of gassing chambers has varied from time to time, the usual type at present consisting of a glass-walled chamber of about 700 to 800 liters with a flow of 200 to 300 liters of air per minute drawn through it by a suction pump, the gas being introduced into the current of entering air. This chamber has certain disadvantages, the principal one being that it takes from two to four minutes to "build up" the desired concentration of gas, and there is a similar period of lag at the end of exposure between the time the gas is turned off and the time when the animal is breathing pure air again. While this defect is of no great importance in prolonged exposures it makes it impossible to accurately time short exposures of one to ten minutes.

In 1924 Professor Haber (Chief of the German Chemical Warfare Service during the late war) told one of the authors that none of the gassing chambers in use at the present time are of any value because the volume of the chamber is so small that the chamber walls and the animal's hair absorb a large amount of the gas, and the rate of gas-air flow is not sufficiently rapid to make up for this defect. Professor Haber predicted that in ten years no chamber would have a capacity of less than 1000 cubic feet and would also have a very rapid rate of flow. It is of interest to note that Lieutenant H. A. Kuhn of the American Chemical Warfare Service had already designed, in 1920, a large gassing chamber which has been altogether satisfactory, and overcomes all such difficulties as lag and absorption of gases by the animal's hair and the chamber walls. The Kuhn chamber is quite complex and in addition is rather expensive, both to build and to operate. It also requires large quantities of the gas to be tested and it is not always possible to obtain these quantities. So although when practicable the Kuhn chamber is the method of choice there are times when it is not feasible to use it.

The chamber we describe was designed in an attempt to overcome the necessity for using the large quantities of gas required by the Kuhn chamber and at the same time to overcome the lag period of older chambers. We do not claim to have designed the chamber as practically everyone that saw it during its development had suggestions to offer, many of which were adopted. In previous chambers the gas has been released upon the animal. In this chamber the animal is quickly introduced into, and at the end of the exposure time, quickly removed from, the gas. The chamber used differs from older methods in that a continuous flow chamber is used, and also in that it has a closed ante-chamber in which the animal is rapidly degassed.

*From the Medical Research Division of Edgewood Arsenal.
Received for publication, June 12, 1925.

Reference to Figs. 1 and 2 will be necessary to explain the construction and operation of the chamber. We will presume that a substance, X, of high boiling point is to be examined.

The subjects are exposed to the toxic effects of the gas-air mixture in the glass-metal chamber *H*. This is an air-tight sealed box of 512 liters capacity, having a detachable front and the necessary entrance and exit holes for the gas-air mixture, and an entrance hole for the analysis tube. Both sides and the top of the chamber are made of glass and the balance of sheet iron. The front side is composed of two sections, an upper and lower part.

By means of the heating units *E* and *E'* made of nichrom wire and controlled by a rheostat, the bubblers *F* and *F'* containing substance *X* are heated to the necessary temperature. Dry nitrogen is then released from cylinder *A*.

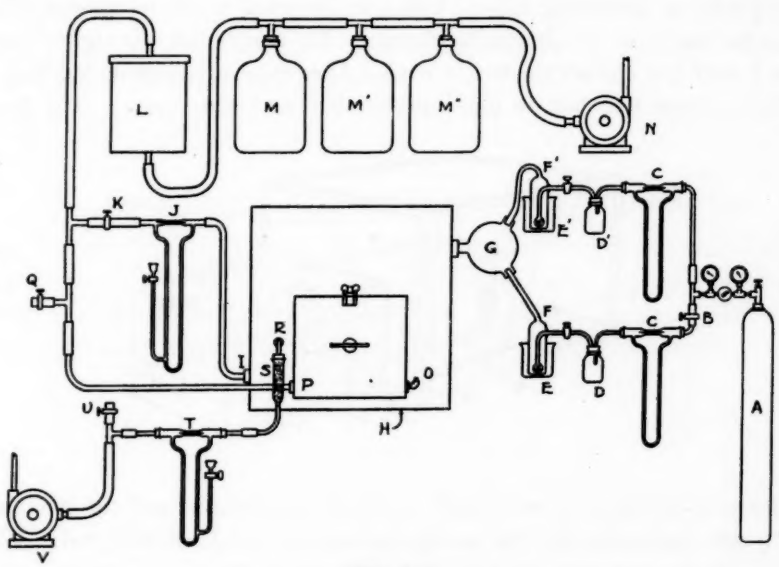

Fig. 1.

The amount of flow of nitrogen is registered by the flow-meters *C* and *C'*, an equal flow through each is controlled by stopcock *B*. *D* and *D'* are traps to catch the overflow from *C* and *C'* in case they are blown. From *D* and *D'* the nitrogen passes through the bubblers *F* and *F'* containing substance *X*. The nitrogen *X* mixture flows at the desired rate of liters per minute. The delivery tubes of the bubblers project into the open ends of the mixing bowl *G* where the gas-air mixture is made just prior to its passage into the gasing chamber *H*. The mixing bowl *G* is a five liter Pyrex flask which has two inlets for the necks of the bubblers, and one outlet which projects into the gassing chamber *H*. The gas-air mixture is drawn into the gassing chamber *H* at *G* and out at *I* at the rate of 200 liters per minute by a size "D" Leimann air pump *N*, capacity 500 liters per minute. The amount of flow through the gassing chamber is registered by the flow-meter *J* connected with *H* at *I* and controlled by stopcock *K*. To prevent the atmosphere surrounding the laboratory from becoming mixed with toxic gas, it is necessary to use a filter *L*. A two inch layer of charcoal

and soda lime in this removes all gas from the effluent air. The large volume of air passing through the line necessitates a series of equalizing bottles *M*, *M'*, *M''* to quiet the fluctuations in the pull of the pump *N*.

Pump *N* not only draws the gas-air mixture through chamber *H*, but at the same time pulls air through the dog box (*b*, Fig. 2) via entrance *O* and exit *P*. The amount is controlled by the by-pass *Q*.

A size B. Leimann air pump *V*, capacity 250 liters per minute, is used to draw gas-air samples from the chamber *H* during the period of exposure through the tube or bubbler *S* filled with a suitable absorbent and inserted in the gassing chamber *H* at *R*. The rate of flow through the tube *S* is registered by the flow-meter *T* and controlled by the by-pass *U*. The usual rate of flow used is ten liters per minute.

Consult Fig. 2, dimensions are given in feet and inches. Entrance to dog box *b* is made by removing lid *c*. Lid *c* is fastened down by means of clamps on all sides, such as *d*, shown in figure. To insure an air-tight connection between *b* and *c* a rubber gasket is used. Dog cage *e* is supported and moved by carrier *g* from dog box to gassing chamber and vice versa. The front end

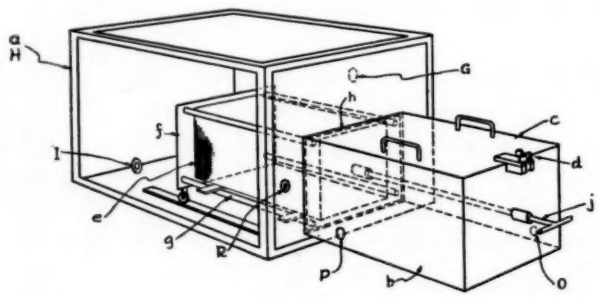

Fig. 2.

of carrier *g* is fastened to rod *j* with a swivel connection, and the movements of carrier *g* are controlled by the manipulation, by hand, of this rod. When the carrier is in the dog box, an air-tight fit is made between rear-end of carrier and rear-side of connection *h* by means of a tongue and groove circuit, and a nut-screw lock on rod *j*. To lock, turn *j* several times to the right. It is necessary that this connection be air-tight to prevent a leakage of gas from gassing chamber into dog box prior to the exposure. When carrier *g* is in the gassing chamber, the front end of the carrier fits plumb against the front side of connection *h*, and is held there by pressure of hand upon rod *j*.

The procedure for exposing a dog is as follows: The dog is placed in the dog box and the dog box closed and sealed tight. Air pump *N* is started and a flow of 200 liters per minute maintained through the gassing chamber. The heating baths *E* and *E'* are turned on. Sample tube or bubbler *S* is fastened to flow-meter *T*, and pump *V* run for a short time to get the desired flow through tube *S*. As soon as the substance *X* has been heated to the desired temperature, the nitrogen is turned on and the gas-air mixture drawn through the chamber to build up the desired concentration. At the end of this period, rod *j* is unlocked by a certain number of turns to the left, and the dog is quickly pushed into the gassing chamber. At the same time pump *V* is started, and

tube *S* inserted into the gassing chamber at *R*. During the exposure pressure is exerted upon the rod *j* by the operator; the flow-meters and heating baths are watched and regulated if necessary.

At the end of the exposure these operations take place simultaneously: the dog is quickly withdrawn from the gassing chamber and rod *j* given several turns to the right; tube *S* disconnected from the gassing chamber and pump *V* stopped; by-pass *Q* is closed. When by-pass *Q* is closed the rate of flow of air through the dog box increases from fifty to over one hundred and fifty liters per minute, which is rapid enough to completely clear it of gas. The sample tube or bubbler *S* is disconnected and its contents analyzed. The analytical result divided by the flow through *S* will give the concentration in milligrams per liter. The dog box is then opened and the dog removed for further examination and observation.

We wish to express our acknowledgment to Lieut. Colonel E. B. Vedder (M. C.), U. S. A., for valuable suggestions and to Mr. Harry Shannon for practical help in building this chamber.

Ohio Society of Clinical Laboratory Diagnosis

The Sixth Annual Meeting of the Ohio Society of Clinical Laboratory Diagnosis will be held in Toledo, Ohio, on the 14th and the 15th of May, 1926, following the meeting of the Ohio State Medical Association. Dr. Jonathan Forman, President, and Dr. C. M. Hyland, Secretary-Treasurer.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Balachowsky, S.: Hemotonia: A New Factor Determining the So-called Sedimentation Velocity of the Erythrocytes. Ann. de Med., September, 1925, xviii, 201.

The author reports his studies of the Fahraeus phenomenon as a result of which he believes that the mechanism of the reaction involves the existence of a previously undescribed factor which he denominates as "hemotonia."

The method used for the test was as follows:

Into a capillary pipette with a diameter of 1 mm., which has first been washed with 5 per cent potassium oxalate, a large drop of blood is drawn from a finger puncture. The column of blood must be from 4 to 10 cm. in height. The pipette is then kept in a vertical position and a reading made, at thirty minutes, of the height of the plasma column above the red cells, from which reading the average hourly velocity of sedimentation can be calculated.

For the detailed study of the mechanism of the reaction the author devised the following technic:

A small receptacle was made by attaching four cover-slips with wax to a slide. The receptacle thus manufactured measured 0.1 to 1 mm. in depth, 10 mm. in height, and 0.5 mm. in width. He suggests that a Burker cell can be used for this purpose.

The receptacle was placed on the stage of a microscope, inclined at an angle of 90°, and filled with oxalated blood.

The reaction was then observed with a micrometric eyepiece.

With the low power there is seen between the mass of red cells and the supernatant plasma, a very distinct and apparently rectilinear line of demarcation. Under the high power, however, this line is found to be irregular, notched, and suggestive of the outlines of a geographical map.

The supernatant plasma shows the presence of rouleaux and conglomerate masses which, after accidental detachment from the general mass of red cells, remain adherent to the walls of the glass cell or seem to be repelled by a current.

These isolated elements fall very rapidly as soon as the masses are freed, accidentally or artificially. Their fall is much more rapid than that of the general mass of erythrocytes.

On examining the general mass of red cells there are no free erythrocytes, all being united in rouleaux which, in turn, often form conglomerate masses. These masses and the rouleaux form a network the spaces of which are filled with plasma. This network has three dimensions and is termed, by the author, the "skeleton of the blood."

The detailed observations described are summarized as follows:

1. The red cells forming rouleaux and conglomerates in the blood constitute a network which is the skeleton of the blood.

2. Under the influence of weight and through a series of local depressions there occurs a general heaping of the blood skeleton on the bottom of the receptacle.

3. This blood skeleton is not deprived of solidarity for: (a) The configuration of a given portion may remain unchanged for a long period of time. (b) The free upper border of the bulk of the erythrocytes preserves its shape for a long period. (c) There exists a force of resistance against the collapse of the blood skeleton other than the viscosity.

4. The solidity of the blood skeleton, being a function of the tonus of its constituents, is the new factor denominated by the author as "hemotonia."

The author believes that these experiments and observations demonstrate that the sedimentation of erythrocytes is not, as has been assumed, merely a simple phenomenon of sedi-

mentation, but a more complicated reaction in which the effects of heaping play an important part. For this reason he would denominate the phenomenon as the "sedi-compilation of erythrocytes."

Rosenow, E. C.: The Precipitin Reaction in the Diagnosis of Scarlet Fever and Allied Hemolytic Streptococcus Infections. Jour. Am. Med. Assn., Jan. 2, 1926, lxxxvi, 9.

Rosenow reports a precipitin test for the diagnosis of scarlet fever and other conditions due to hemolytic streptococci.

The immune scarlatinal serum was prepared by the intravenous injection of a horse with freshly isolated hemolytic streptococci obtained from scarlatinal sore throats.

Small tubes are made from thin-walled glass tubing having an inside diameter of 3 to 3.5 mm. cut into lengths of 3.5 cm. and sealed at one end.

A capillary pipette made from the same tubing with a capillary outside diameter of about 0.5 mm. is used to add the extract and serum.

Material for extraction is secured by swabbing the nasopharynx, contact with the tongue being scrupulously avoided.

The swabs are made of absorbent cotton wrapped around the roughened end of aluminum wire (1.5 mm. diameter). The swab is then bent at an angle of 60°. The extraction may be made immediately or after the swab has dried while kept in a sterile tube.

The swab is placed in 2 c.c. of sterile normal saline or in 0.2 per cent gelatine Locke solution and thoroughly agitated. The cotton, removed from the end of the wire with forceps, is expressed by wrapping it around the end of the forceps while pressing it against the wall of the tube.

The resultant mixture is cleared by centrifugation at high speed in narrow tubes. The clear fluid is taken up with the capillary pipette, the end of which is then placed at the bottom of the precipitin tube and enough of the fluid ejected to make a column about 1 cm. in height. The pipette is then rinsed in three changes of normal saline and an equal volume of immune serum added, the tip of the pipette being again placed at the bottom of the tube so that the serum lies beneath the extract.

Extract and serum should be water clear or, at most, only slightly opalescent

The tubes are incubated two hours and then read and a second reading is made after standing in the ice chest overnight. The two readings usually agree.

Readings are best made in a darkened room by transmitted light.

Negative: absence of cloud at zone of contact.

Plus-one: slight cloud.

Plus-two: moderately heavy cloud.

Plus-three: heavy, slightly granular cloud.

Plus-four: heavy cloud with precipitation.

Robin, A.: A New Method for Enriching the Stools. Compt. rend. Soc. de Biol., Paris, May 1, 1925, xlii, 1099.

1. Introduce a piece of feces about the size of a nut into a flask containing about 20 glass beads and 14 c.c. of Schweitzer's reagent (formula not stated). Mix thoroughly and rapidly.

2. Pour off the liquid obtained into two centrifuge tubes and centrifuge for one minute.

3. Pour off the supernatant fluid and wash the outside of the tubes, held vertically, by rapid agitation in water without disturbing the sediment. Add 4 c.c. of normal saline and agitate thoroughly.

4. Filter through a metallic sieve with a mesh of 340 microns.

5. Add 4.5 c.c. of a mixture of HCl 22 parts, formol 34 parts, and normal saline 44 parts. Shake for fifteen seconds. Add 12.5 c.c. of ether and shake vigorously for about fifteen seconds.

6. Divide the mixture into two centrifuge tubes and centrifuge one minute.

7. Examine the sediment. If cysts are not seen, it is necessary to remove with a pipette a little of the mixture at the junction of the ether with the fluid.

Ova and cysts are but very slightly changed in form and structure.

Nadler, W. H., Starr, P., and Tukey, G.: Glycemia as a Guide to the Treatment of Diabetes Mellitus. Arch. Int. Med., October, 1925, xxxvi, 579.

It is generally recognized that repeated blood-sugar estimations are an invaluable if not, indeed, an essential feature of the treatment of diabetes. The development of micro methods for the determination and of means for the preservation of the blood samples has greatly facilitated such studies but the necessity for repeated visits to the laboratory by fasting patients is still a source of annoyance.

The authors, after a study of various micro methods and methods for the preservation of the sample, have devised a procedure whereby blood can be collected by the patient, preserved, and forwarded to the laboratory as convenient.

For the collection of the sample small, wide-mouthed vials (7 × 12 mm.), fitted with a rubber stopper were utilized. In each bottle is placed 5 mg. of a 10:2 finely powdered mixture of sodium fluoride and thymol.

Patients were supplied with an automatic spring lancet, the point of which had been sharpened to resemble a Hagedorn needle, and taught how to prick the finger and milk approximately 0.2 c.c. of blood into the vial. The blood and the chemicals were then mixed with a toothpick, the vial stoppered, and sent to the laboratory by mail.

About 8 drops of blood was sufficient.

Samples so collected showed no marked loss in blood sugar, for as long as eleven days, when examined by a micro method (that described by Randles and Griggs, Jour. Am. Med. Assn., Mar. 1, 1924, lxxxii, 684).

Soldin, M., and Lesser, F.: Study of Induced Serum in Syphilitic Infants. Deutsch. med. Wchnschr., Oct. 30, 1925, li, 1817.

The authors report the consistent demonstration by dark-field illumination of Spirocheta pallida in the serum from blisters induced by cantharidin-collodion on the skin of syphilitic infants in whom syphilitic eruptions were present.

The method was not successful in adults.

Boyers, L. M., Kofoid, C. A., and Swezy, O.: Chronic Human Amebiasis. Jour. Am. Med. Assn., Nov. 7, 1925, lxxxv 1441

The authors, in a well illustrated paper, report a review of the diagnosis and treatment of chronic human amebiasis on the basis of encystment in the liver area.

They recall that it is a common inference that encystment of E. dysenteriae occurs only in the colon and call attention to the fact that it is logical to believe that an early, mild hepatitis is a concomitant or sequel of the mild colitis occurring as a characteristic sign of amebiasis in the majority of cases.

Microscopic studies, which are illustrated in the paper, bring abundant evidence of entrance of amebae into the blood stream from whence they may pass to the liver through the hepatic portal stream. The hematogenic distribution of amebae from colonic infection is thus provided for and, in the opinion of the authors, occurs more frequently than has hitherto been recognized.

This they believe to be responsible for the existence of chronic amebiasis as a clinical entity.

The necessity for a careful and minute history is emphasized, salient symptoms being fatigability and "bowel consciousness."

They emphasize that the condition may be mistaken for a variety of diseases.

To detect hepatic colonization by amebae they resort to duodenal drainage according to the method outlined below.

The patient is examined without breakfast. The ordinary Rehfuss duodenal tube is passed and the patient placed on a couch on his right side. A group of four flasks is made ready. Into flasks 2 and 4 is placed approximately 50 c.c. of Bouin's solution. (Saturated aqueous solution picric acid 75 parts; glacial acetic acid 5 parts; formalin 20 parts.)

The end of the tube is placed in flask 1 and allowed to remain there until the fluid begins to siphon away. When the fluid in flask 1 begins to show a tinge of bile, the tube is

passed into flask 2 and allowed to drain for about one hour. Then 125 c.c. of warm saturated solution of magnesium sulphate is introduced with a glass syringe and as much of the solution as possible is withdrawn by the syringe. The tube is then allowed to drain into flask 3 until all the magnesium sulphate is passed and dark cystic bile begins to flow, when the tube is allowed to drain into flask 4.

The so-called hepatic bile may, if desired, be collected in a fifth flask containing the same fixative solution.

The material in flasks 2 and 4 is then centrifuged and the sediment, the cellular part of which is already fixed, stained directly or the sediment may be infiltrated in paraffin, sectioned, and stained and then examined.

By this method encysted amebae may often be demonstrated.

Sturtevant, M.: Achlorhydria Preceding Pernicious Anemia. Jour. Am. Med. Assn., Nov. 21, 1925, lxxxv, 1638.

The absence of HCl in the gastric contents is a common finding in pernicious anemia and the possibility of its causal relation to this condition has been considered.

Sturtevant reports a case of known achlorhydria of fourteen years' duration prior to the onset of the symptoms of pernicious anemia.

The symptoms of achylia began in 1899; the diagnosis of achlorhydria was made in 1900 and the symptoms of pernicious anemia detected in 1916.

He believes the case to be the longest as yet recorded.

Lederer, M.: A Form of Hemolytic Anemia Probably of Infectious Origin. Am. Jour. Med. Sc., October, 1925, clxx, No. 4, 500.

Lederer reports the observation of three cases characterized by sudden onset with elevation of temperature, leucocytosis, and a severe anemia resembling pernicious anemia, all of which symptoms promptly and rapidly disappeared following a single transfusion.

He comments upon the paucity of similar clinical observations and believes the condition not infrequent but, perhaps, obscured by unfamiliar terminology.

The outstanding features are the acuteness of the symptoms, evidence of very rapid erythrocytic destruction, extreme regenerative action of the bone marrow, rapid recovery after a single transfusion, and the absence of sequelae.

In all of the cases the stained smear showed a very close resemblance to the picture seen in pernicious anemia during a crisis. Because of the large number of erythroblasts, the leucocyte count was corrected by subtracting from the number of white cells estimated by the white cell pipette, the number of erythroblasts estimated from the stained smear.

The leucocyte counts varied from 25,800 to 52,000 with 51 to 85 per cent polymorphonuclears. Enormous numbers of erythroblasts were found, 3000 per c.mm. in one instance.

Red cell fragility was normal in all cases and all the usual laboratory examinations were negative.

The spleen and liver were enlarged in two cases and the eyegrounds in two cases showed recent changes of a type commonly found in pernicious anemia. Bone marrow tenderness was absent.

The leucocyte count is tentatively explained as possibly due to three factors: infection, increased production due to the stimulation of a toxin, and heaping up. The nature of the infection or the character of the toxin remain unknown.

Bott, C.: The Diagnosis of Cancer in the Blood Serum. Münch. med. Wchnschr., Nov. 13, 1925, lxxii, 1959.

Bott reports his experience with the serologic test for cancer devised by Wigand.

Blood is secured by venipuncture, chilled, and the serum removed. The test should be made promptly after securing the specimen.

A series of dilutions of the serum with normal saline is then set up in twenty-four tubes, the dilutions increasing in "geometrical progression." The final volume in each tube is 2 c.c.

To each tube is then added 1 c.c. of a freshly prepared and filtered 1 per cent

solution of tannic acid and two or three drops of a "strongly diluted" solution of carbolfuchsin, the purpose of which is to render flocculi more visible.

Readings are made of the degree and extent of flocculation at twelve, eighteen, and twenty-four hours.

In normal cases flocculation occurs to Tube 12 at twelve hours, to Tube 16 at eighteen hours and to about Tube 18 at twenty-four hours. The reaction is negative if there is no flocculation beyond these points. Carcinoma "of slight extent" gives a reaction similar to that seen in normal cases; advanced carcinoma, and also late pregnancy, show flocculation to the limit of the serum dilutions.

Bott considers the reaction a lability reaction, nonspecific, and of dubious value, disagreeing with Wigand in these respects.

Patten, A. C.: The Human Cerebrospinal Fluid in System and Diffuse Degenerative Disease Involving the Nervous System. Amer. Jour. Med. Sc., Jan., 1926, clxxi, No. 1, 48.

The author tabulates, from the literature, a large number of group studies made in a variety of conditions.

The findings in some of the more frequent conditions are thus summarized:

Multiple Sclerosis:

Pressure: no significant variations.

Protein: increased in 50 per cent of cases; no uniformity.

Cell count: 0 to 42 per cu. mm.; no pleocytosis in 75 per cent of cases.

Colloidal gold: inconstant, ranging from negative to luetic reactions.

Sugar: no uniformity.

Creatinin: 1.78 mg. per 100 c.c.

Urea: 15.5 mg. per 100 c.c.

The Encephalitides:

Pressure: variable, generally increased.

Protein: increased (72 per cent of cases).

Cell count: may vary from normal to several hundred; mononuclears predominate; greatest number of cells in the first three weeks, then declines.

Sugar: ranges from 0.062 to 0.095 mg. per 100 c.c.

Results of examination are constant only in inconsistency.

Poliomyelitis:

Pressure: normal or slightly increased.

Protein: slight to moderate increase.

Cells: early high count: lymphocytes and large mononuclears predominate; later variable to normal.

Wassermann: negative.

Colloidal gold: luetic zone reaction.

Sugar: increased.

Epilepsy:

Pressure: normal.

Cells: normal.

Globulin: negative to slight increase.

Wassermann: negative.

Colloidal gold: negative.

In the encephalitides the Wassermann is negative and the colloidal gold reaction inconstant, varying from luetic to paretic curves.

Numerous other neurogenous, traumatic, infectious, and degenerative conditions are reported, the results being very inconstant.

Plass, E. D., and Mathers, C. W.: Placental Transmission III: The Amino Acids, Nonprotein Nitrogen, Urea, and Uric Acid in Fetal and Maternal Whole Blood, Plasma, and Corpuscles. Bull. Johns Hopkins Hosp., June, 1925, xxxvi, No. 6, p. 393.

In view of the discordant findings reported in the literature, this question was restudied by the authors. The methods of Folin and Wu were used throughout on specimens obtained from the maternal arm vein and the umbilical cord.

The amino acids are uniformly higher in normal fetal whole blood and plasma than in the maternal blood and plasma.

The total nonprotein nitrogen is always higher in the fetal blood. Urea is usually the same in both bloods.

Uric acid is generally the same; if there is a difference, it is generally higher in the fetal blood.

The article contains numerous tables.

Isaacs, R.: Effect of Roentgen Ray Irradiation on Red Blood Cell Production in Cancer and Leucemia. Amer. Jour. Med. Sc., Jan., 1926, clxxi, No. 1, p. 20.

It is generally accepted that small doses of x-rays have a stimulating and large doses a depressing effect upon living tissues, the latter effect being due to tissue death.

Isaacs endeavored to study the mechanism of the depressant action by determining the variations in the red cell count after therapeutic irradiation in cancer and leucemia. Attention was focussed particularly upon "granule red cells," mature corpuscles containing a single refractive granule 0.5 micron in diameter and representing an intermediate stage between the reticulocyte and the normocyte, and the reticulocytes.

In health these corpuscles vary from 1 to 3 per cent. An increase above three per cent usually means abnormal blood production. Variations above three per cent are of more significance than decreases below this figure. The cells are only demonstrable by vital staining.

Blood was examined before and after irradiation in a series of 30 patients (carcinoma, 17; leucemias, 10; erythremia, 2; and chronic hemolytic jaundice, 1).

Blood films were stained supravitally with cresyl blue, using Wright's stain as a counterstain.

Differential counts were made of 1,000 to 5,000 red blood cells.

The degree, rate, duration, and site of radiation varied in accordance with the case.

In susceptible cases irradiation stimulated changes which are normal in the life of a red blood cell, only the rate and time of initiation being varied.

Following effective short-ray radiation, in one to twenty-four hours one of several responses may be noted. The granule cells may show an increase, appreciable or negligible, temporary or sustained.

Where the increase is relative or absolute there may be a relative or absolute decrease in reticulocytes unless the marrow is hyperplastic (hemorrhage).

Where the increase of granule cells is significant and sustained (two or more days), definite clinical improvement is seen in cancer and leucemia, the reverse being also true.

Where the increase is temporary and followed by a leucopenia, there has been too much radiation and the patient may become worse clinically.

The most frequent effect of radiation is an increase, gradual and temporary, in the number of red cells per cu. mm. and an increase in the color index which may rise above one.

The change in the percentage number of immature red blood cells is one of orderly stimulation to reach maturity.

The percentage changes of immature cells appears to be a systemic effect of localized exposure and the changes in the relative percentages are similar to those following transfusion.

The period between the application of irradiation and the beginning of maturation changes is probably a matter of minutes as compared to hours for the leucocytes and weeks for tissue cells.

From the evidence of the effect upon young red blood cells, all roentgen ray irradiation may be considered as stimulative: small doses stimulate to division; large doses may stimulate to maturity and death by senility, the rate for each tissue varying with its specific growth properties.

Brown, G. T.: Bacterial Vaccines in Asthma. Amer. Jour. Med. Sc., Jan., 1926, clxxi, No. 1, p. 94.

There has been so much discussion regarding the use of bacterial vaccines in asthma that Brown details a method of diagnosis and treatment which has given very good results in his hands.

A careful and minute history and physical examination are essential preliminaries upon which he justly lays great emphasis.

These are followed, *when indicated*, by such special examinations as x-ray, when they seem advisable and then by skin tests with such pollens, proteins, and bacterial proteins as may be placed under suspicion by the history. He does not believe in the indiscriminate testing of everybody with everything and calls attention to the importance of looking for delayed reactions.

When the leucocytes are over 10,000 and the polymorphonuclear count above 80, a careful search is made for a focal infection. Lymphocytosis calls for the elimination of tuberculosis, lues, or a latent streptococcic infection. A Wassermann is made in all cases of continuous asthma as many of these will clear up under antiluetic treatment.

He does not agree with the importance given to asthma as a cause of eosinophilia and, when this is present, advises a careful search for other causes.

When all investigations have failed, he assumes that the case has been shown, by elimination, to be due to bacteria. Cultures are then made of the sputum, organisms isolated in pure culture, and a vaccine prepared from each, containing two billion organisms per cubic centimeter. Intradermal skin tests are then made with each vaccine, injecting enough to produce a blister ¼ inch in diameter and using as a control normal saline containing a trace of agar as this is generally present in the vaccine.

Only those vaccines are used for treatment to which the patient reacts. With this method very favorable results have been obtained.

Kubie, L. S., and Schultz, M. G.: Vital and Supravital Studies of the Cerebrospinal Fluid and of the Meninges in Cats. Bull. Johns Hopkins Hosp., August, 1925, xxxvii, No. 2, p. 91.

The investigation reported was concerned with a study of the varied cell pictures seen in the cerebrospinal fluid and commonly associated with imflammatory processes, with particular reference to "pus cells," "macrophages," and "fibroblasts"; their origin, and significance and how far they may be capable of transformation from one form to another.

The difficulty in the cytological study of stained smears of the spinal fluid in inflammatory processes has been that the slow evaporation of the large amount of watery fluid in which the cells are suspended has produced a marked shrinkage and distortion.

In these studies the essential data were secured from a study of the living cells. A new method was also devised for the fixation of cells without much distortion, which is described below.

A strip of glass is sealed to the bottom of a Petri dish with a little balsam. A few crystals of iodine are scattered loosely over the bottom of the dish, which is warmed gently just before using, until faint purple fumes fill the chamber.

A coverslip, containing a single large drop of spinal fluid, is then placed on the strip of glass in the dish.

The cells are fixed almost at once. The drop itself dries quite slowly but when it is dry the cells have settled down upon a small circular area of the slip, firmly attached to the glass surface.

The preparation is now deeply stained with minute crystals of iodine and is overlaid with a fine film of coagulated protein.

The excess iodine of this preparation is washed out with a strong aqueous solution of potassium iodide, after which it is rinsed in water and stained. The authors used Mayer's carmalum to secure a contrast with trypan-blue inclusions, which substance they used to produce the inflammatory reactions, and for a more detailed study of the cell morphology, the Unna-Pappenheim combination of pyronin and methyl-green.

For the study of living cells the method described by Sabin, was utilized (Bull. Johns Hopkins Hosp., Oct., 1921, xxxii, 368; ibid. Sept., 1923, xxxiv).

The paper is very detailed in its discussion of the observations made and illustrated and should be read in the original.

The results are thus summarized:

In the first twenty-four to forty-eight hours after the onset of meningeal irritation,

polymorphonuclear leucocytes predominated, being later usually outnumbered by mononuclear forms.

In relatively chronic inflammations lymphocytes predominate and appear to accumulate in the perivascular channels, being partly released by drainage of the spinal fluid and more competely washed out by the simultaneous intravenous infusion of hypotonic solutions. This procedure may have possible therapeutic applications.

It is suggested that the so-called plasma cell is identical with the intermediate lymphocyte. Phagocytes arise from young phagocytic forms normally present throughout the meninges.

The cerebrospinal fluid accelerates the degeneration and inhibits the motility of the polymorphonuclear neutrophilic leucocytes and the lymphocytes to a marked degree. It is less destructive to the eosinophilic leucocyte and the phagocyte.

Lipshutz, B., and Lowenburg, H.: Pneumococcic and Streptococcic Peritonitis: Report of Twenty-three Cases in Infancy and Childhood. Jour. Am. Med. Assn., Jan. 9, 1926, lxxxvi, 99.

Commenting upon the high mortality, practically 100 per cent, of this condition, the lack of efficient means for its treatment, and the fact that it is not as infrequent as has been supposed, constituting about 10 per cent of the acute abdominal emergencies of childhood, Lipshutz and Lowenburg suggest the necessity for its serious study.

They report twenty-three cases occurring within three years.

The disease appears to be idiopathic. The streptococcus was usually of the hemolytic variety. Typing of the pneumococcus was not done. None of the pneumococcic cases showed any pulmonary lesion, as demonstrable by physical examination or radiography.

They consider it very significant, however, that a history of mild upper respiratory or pharyngeal infection was present in every case in which the peritonitis was of pneumococcic origin.

The authors believe that there is a distinct relation between coryza or throat infections and pneumococcic or streptococcic peritonitis.

They review the four possible portals of entry:

1. The blood stream. In nine cases a positive blood culture was secured.

2. The lymphatic tract, which seems improbable and conflicts with the pathology of the condition.

3. By the genital tract. Pneumococcic peritonitis is most common in girls (3:1). The series reported shows that, with one exception, all the pneumococcic infections were in girls.

The pneumococcus was isolated from the vaginal secretions, peritoneum, and blood in three cases. The peritonitis usually begins as a pelvic peritonitis. The disease is most frequently encountered where there are unhygienic conditions. For these reasons the authors believe that the genital tract may be an important source of infection in this disease.

4. The gastrointestinal tract also fits in with the clinical and pathologic studies of this disease and gastrointestinal disturbance was present in every case. It is difficult, however, to produce peritonitis experimentally by using the gastrointestinal tract as the experimental portal of entry.

The paper discusses in detail the relation of appendicitis in infancy to pneumococcic and streptococcic peritonitis.

Peritoneal puncture and examination of the exudate by smear and culture is advocated as a diagnostic measure. Untoward results from this procedure were not encountered.

The clinical picture is described in detail.

A serious study of this infection by the pediatrician, interne, surgeon, and laboratory worker is urged.

Birkhaug, K.: Demonstration of Antigenic Relationships Among Strains of Streptococcus Erysipelatis by Intradermal Protection Tests. Bull. Johns Hopkins Hosp., Aug., 1925, xxvii, 2 to 85.

This paper is one of a series of studies on the biology of Streptococcus erysipelatis and reports a method whereby these organisms may be differentiated from the group of streptococci associated with scarlet fever and numerous other streptococcic infections.

The study reports the results of intradermic tests conducted on rabbits.

An area 8 x 12 cm. was depilated on both sides of the chest and abdomen and then divided into smaller areas approximately 4 cm. in diameter. These small areas were then infiltrated with 0.1 c.c. of immune sera (erysipelas serum prepared by the immunization of rabbits, scarlatinal antistreptococcic serum, and heterologous antistreptococcic serum). After an interval of twenty-four hours to allow fixation of the immune bodies, into the center of each area thus passively immunized 0.1 c.c. of a hemolytic streptococcus emulsion from a 24-hour blood agar slant suspended in 10 c.c. of Locke's solution was injected intradermally.

The pathogenic activity of Streptococcus erysipelatis was inhibited only by the erysipelas serum. Nonspecific protection was not afforded by any of the other sera nor by normal rabbit serum.

By this method of local passive immunity it was possible to differentiate a group of hemolytic streptococci causing erysipelas from a group of hemolytic streptococci causing scarlet fever on the one hand, and from the large series of miscellaneous hemolytic streptococci producing a variety of pyogenic infections on the other.

Rienhoff, Jr., W. F.: Histologic Changes Brought about in Cases of Exophthalmic Goiter by the Administration of Iodine. Bull. Johns Hopkins Hosp., November, 1925, xxxvii, No. 5, p. 285.

The administration of iodine in the treatment of exophthalmic goiter produces both gross and microscopic changes in the gland, which have been studied and are reported by Rienhoff in an excellently illustrated article.

The tissues were Zenker-formalin fixed and stained with hematoxylin-eosin.

The histologic changes produced by iodine are thus summarized:

(a) An increase in the size of the thyroid gland as a whole.

(b) A decrease in the vascularity and probably in the lymph flow throughout the gland.

(c) A large increase in the amount of fibrous connective tissue.

(d) A striking increase in the amount of colloid deposited and contained within the thyroid gland.

(e) A change in the acini from lace-like papillomatous ingrowths to round, even-walled, smooth acini, regular in size and form.

(f) A transition of epithelium from high columnar to flat cuboidal and occasionally low columnar.

(g) A change in the nuclei of epithelial cells from large clear nuclei to the small, irregular, pyknotic type.

(h) The presence of many mitotic figures before the use of iodine and their absence after iodine has been given.

The histologic picture following the use of iodine appears to be constant.

Mikulowski, W.: Diagnostic Value of Bacilli of Koch in the Feces of Children. Arch. de med. des Enf., April, 1925, xxviii, 201.

In view of the fact that it is difficult to secure specimens of sputum from children, by whom sputum is frequently swallowed, the author examines the feces for tubercle bacilli, using the technic described by Besancon and Jong.

To about 30 grams of feces in a sterile glass sufficient 25 per cent solution of sodium cholride is added to make a semiliquid mass by trituration. The mixture is then filtered into two centrifuge tubes through sterile gauze, 2 c.c. of a mixture of equal parts of ether

and ligroin added to each tube, the tubes stoppered, thoroughly shaken, and centrifuged for ten minutes at 5000 r. p. m. Immediately below the ether layer is a cake about 2 mm. thick in which the tubercle bacilli, if present, will be found. Smears are made from this cake and stained by the usual methods.

Frei, W.: A New Skin Reaction in Inguinal Lymphogranuloma. Klin. Wchnschr., Nov. 5, 1925, iv, No. 2, p. 48.

In the course of a study of five cases of this condition the author devised the skin test detailed below.

From a soft focus in a bubo, pus was aspirated and diluted 1:10 with normal saline and the mixture heated for two hours at 60° C. and again, on the following day, for one hour at the same temperature. It was then cultured for sterility.

Into two patients and three normal controls, 0.1 c.c. of the diluted sterile suspension of pus was injected intracutaneously in the deltoid region.

In twenty-four hours only a slight reddening at the site of the injection was observed in the controls. In the lymphogranuloma cases, however, there was an inflammatory reaction 0.75 to 1 cm. in diameter associated with the formation of papules. The reaction increased in intensity in thirty-six to forty-eight hours, when the inflamed area measured 2 to 3 cm. in diameter, and then gradually subsided with slight superficial necrosis.

Repetition of the test gave similar results varying somewhat in intensity in accordance with the source of the antigen.

Complement-fixing bodies were not detected.

There was no difference between the controls and the cases when injected with pus similarly prepared and obtained from staphylococcal infections, tuberculous glands, or material from tuberculous glands, doubtful malignant granuloma, syphilitic liver extract, or Ducrey bacillus vaccine.

The antigen from lymphogranuloma cases gave no reaction in patients with other types of gland infections, such as: syphilis, tuberculosis, malignant granuloma, lymphatic leucemia, or lupus.

It is suggested that the reaction is due to the presence in the pus of either the etiologic agent of the disease or its elimination or disintegration products.

If the reaction proves constant in a large number of cases, the author suggests that it may be of aid in differentiating lymphogranuloma inguinal from other localized glandular infections.

Wohl, M. G.: Hemophilia. Neb. State Med. Jour., Nov., 1925, x, No. 11, p. 442.

Wohl, in a case report, recalls the efficiency of a method of treatment which he first heard suggested by Da Costa.

A full grown rabbit is anesthetized; the liver is removed under aseptic precautions and ground in a sterile meat grinder.

About 3 c.c. of sterile normal saline is added for each gram of liver substance and the mixture allowed to stand for forty-five minutes to permit extraction of thrombokinase. The mixture is then filtered. Cotton saturated with the filtrate is applied as a dressing.

Sabin, F. R., Cunningham, R. S., Doan, C. A., and Kindwall, J. A.: The Normal Rhythm of the White Blood Cells. Bull. Johns Hopkins Hosp., July, 1925, xxxvii, No. 1, p. 14.

The sudy reported concerns itself with the mechanism involved in the remarkable constancy maintained in the number of leucocytes in the circulating blood.

Through the use of a supravital staining technic the number of white cells, their death and replacement, was found to vary in a definite rhythm. The staining method has been published by Sabin (Bull. Johns Hopkins Hosp., 1923, xxxiv, 277).

The article abounds in tabular statistics and in a detailed discussion of the various forms of white blood cells seen in the circulating blood. The results of the study may thus be summarized:

1. There is a characteristic rhythm of the total white blood cell count with an interval of approximately one hour's duration.

2. The total number of white blood cells varies in the proportion of 1:2.

3. There is an afternoon increase in the total white cell count, irrespective of the ingestion of food and due entirely to an increase in the number of polymorphonuclear neutrophiles.

4. The polymorphonuclear neutrophiles die out in showers, often of considerable proportions, the dead cells being promptly replaced from some reservoir as yet undetermined.

5. The lymphocytes have a shorter rhythm than the leucocytes, but the total number varies as 1:3 in contrast to 1:2 for the leucocytes.

6. A small proportion of monocytes are normally undergoing division in the circulating blood.

7. In five of six apparently normal individuals, small percentages of myelocytes were found in the circulating blood.

The paper contains a wealth of detail which does not lend itself readily to abstracting and should be read in its entirety by those interested in the subject.

Faupel, M. H.: A Comparison of the Kahn and Wassermann Tests. Bull. Johns Hopkins Hosp., Sept., 1925, xxxvii, 3 to 170.

A series of comparative tests on 400 patients is reported.

The Kahn test was performed by the procedure in vogue and described in 1923 by Fox and Sanderson (Amer. Jour. Syph., 1923, vii, 687); the Wassermann test was performed with a cholesterinized heart extract and cold incubation, no other details being given.

In general the coefficient of agreement was 0.79, corresponding to 90 per cent absolute agreement.

The results with spinal fluid were not nearly so favorable as those reported by Kahn.

The Kahn test is regarded as a valuable corroborative check on the Wassermann; but is not considered as replacing the complement-fixation test in the serologic diagnosis of syphilis.

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EDITORIALS

The Argentaffine Tumors of the Appendix. What Are the So-Called Carcinoid Tumors?

PRIMARY epithelial tumors of the appendix have been reported with increasing frequency during the past few years. Considerable doubt has been raised as to the character of many of the early cases which were reported. In 1897 Letulle and Weinberg, and Mossé and Daunic reported in rapid succession primary epithelial appendiceal growths which did not correspond in clinical course to carcinoma, and showed no tendency to recur after removal of the appendix.

Statistics indicate that primary tumors of the appendix are rare. Williams, in studying 15,481 tumors, among which were 7,878 carcinomas, found no tumors of the appendix. Kelynack has stated that primary tumors of the appendix are practically unknown. In more recent statistics dealing with diseases of the appendix one cannot help being struck with the rather constant percentage with which the so-called primary carcinoma of the appendix is

found in a large series of appendices removed during the first or recurring attacks of appendicitis. Kelly reports in his series of 706 cases, two (or 0.3 per cent) cases of so-called primary carcinoma; A. and E. Moschcowitz, six (or 0.3 per cent) in 2,000; and McCarthy and McGrath, 22 (or 0.4 per cent) in 5,000. These rather constant percentages in different series of large numbers of cases would indicate that they were dealing with a lesion different from carcinoma. The rather constant percentage would suggest that this lesion might be found in any series in which the appendix was removed because of an inflammatory process or as an incidental finding in appendices removed during the course of other abdominal operations.

These growths are rarely multiple, but similar growths in the small intestine not infrequently are. The shortness of the appendix may account for the rare multiplicity. In a number of cases the appendix, the seat of such a growth, removed during an operation for some other abdominal lesion has been practically normal in appearance. In most of the cases, however, the appendix has been the seat of an acute or chronic inflammatory process. Frequently it is more or less adherent; at times the appendix is adherent only at the site of the tumor.

The form of the appendix depends upon whether there is kinking or torsion. The tumor is most frequently situated in the tip or distal third of the appendix. The appendix the seat of such a growth has a bulbous appearance, the bulbous enlargement often appearing nodular. In many of the cases that have been observed the epithelial growth has been found in a scar, or in a dilated portion distal to an obliterated section of the appendix.

The tumor may be so small that it escapes the naked-eye examination of the pathologist or surgeon, and a microscopic examination gives the first indication of the presence of such a growth. In most cases, however, the tumor is suspected because of the bulbous enlargement of the tip of the appendix, or a peculiar retraction or induration. Upon section the growth has a shiny, frequently transparent appearance. It is of firm consistency. The color is striking. While in many cases it is a grayish white, in the majority it is yellow, brownish, or even orange yellow in color. At times the growth appears caseous, suggesting a tuberculous lesion. At times the mucous membrane covering the growth appears yellow in color. This yellow appearance is due to the rich lipoid content of the cells and is of considerable diagnostic significance.

The submucosa is the principal seat of these growths. They are not, however, always sharply delimited. The changes in the mucosa vary considerably. It is usually thinner than normal, the glands are decreased in number, and degenerative changes are noted in the cells. The mucous membrane may be reduced in thickness, the cylindrical cells being flattened. Ulceration may occur in the late stages. When the growth is circular the lumen of the appendix may be obliterated. This may occur when the growth is confined to a portion of the wall of the appendix. As a rule there is no similarity between the tumor cells and those of the mucous membrane. Mossé and Daunic believe however that they have found a direct relationship, and Rolleston and Jones believe that genetically they are related. Hart has reported a case in which the glands

of the mucosa present a peculiar atypical growth, in parts resembling a carcinoma without infiltrating characteristics. The principal part of these growths always lies in the submucosa. The stroma varies in amount and kind in different cases and in different parts of the same tumor, transitions from a loose cellular to thick connective tissue with few cells being common.

Oberndorfer believes that an independent muscular stroma is peculiar to these growths, and Saltykow regards the muscle tissue as specific.

Forbus, in a recent contribution on this subject, states that the total number of cases of appendiceal carcinomata found in the literature up to 1923 was 317, and to this number have been added several recent cases. It would seem, therefore, of little importance to place on record further cases, all of which are characteristic and similar in every respect to those already described. And so, were it not for the fact that the true pathologic character of the primary tumors of the appendix and small intestine has been of late the subject of such widespread interest, and their clinical and pathologic characteristics the subject of such varying interpretation, a report of further cases would hardly seem justifiable.

One finds in the literature the following opinions expressed concerning the nature of these growths:

1. The tumor is a carcinoma, originating in the epithelium of the appendix.
2. That they are analogous to the basal cell tumors occurring in the skin.
3. That they develop from pancreatic rests or represent adenomyomas.
4. That they develop from the chromaffine cells of the crypts of Lieberkühn and are endocrine tumors developing from the paraganglionic system.

The following histologic description given by Forbus gives so well the character of the growth that it is given in toto, for the diagnosis can be made upon the grosser characteristics of the cells and their arrangement without resorting to the finer methods.

"The tumor tissue is composed of strands and small masses of round or oval cells embedded in an eosin-staining hyaline stroma. Almost all of the nests and strands of cells lie in the supporting tissue of the tumor but none of them contain tumor cells. The more one studies these spaces filled with tumor cells, the more one is impressed with their lymphatic-like appearance. In places flattened spindle cells exactly like endothelium are seen lining the spaces, although no place can be found where the spindle cells form a complete sheath about the tumor mass. No blood cells are found in contact with the tumor cells. Sometimes the cells distribute themselves about the space in such a way as to form an alveolar-like structure with a rather atypical and irregular lumen. This appearance seems, however, to be only an artefact. A true gland-like arrangement is quite definitely not present. In the more solid nests of cells no alveolar arrangement whatever is seen. The cells of the tumors are not confined entirely to the nodular mass in the appendix; a few strands and cell nests can be seen in the circular layer of muscle. The tumor is, therefore, not encapsulated but has very invasive and infiltrative characteristics. The cells which compose the tumor are round and oval in shape. The nuclei are round, comparatively large, quite regular in shape, uniform in size, and rich in chromatin which is distributed diffusely, though coarse granules may be

found in almost all of the nuclei. Nucleoli are quite frequently seen. The cytoplasm is abundant in some cells, but in the greater part of the tumor it is only moderate in amount in comparison with the size of the nucleus. Coarse granules, which have a rather reddish brown color, are found in most of the cells. Cytoplasm that contains no granules, on the other hand is full of vacuoles. Nowhere can cells be found which have a clear-cut columnar form, like the cells of the crypts of Lieberkühn.

"The stroma which forms the greater part of the tumor nodule consists largely of hyaline connective tissue. Small blood vessels are fairly common, but there are a few larger ones. Nerve fibers are numerous and present an interesting relationship to the tumor cells. In places the cells form an interrupted sheath about the nerve fibers. Toward the periphery of the nodule can be seen small groups of smooth muscle cells which are continuous with the circular coat of muscle surrounding the tumor. No muscle can be found with certainty within the tumor mass, although there are numerous isolated groups of spindle cells which morphologically are like smooth muscle. These do not, however, react characteristically to the differential stains. Nowhere can there be found any supporting stroma within the cell nests and strands. The cells are simply packed loosely together without any intervening tissue. Isolated groups of tumor cells and sometimes single cells can be seen far out in the muscle coat and in what appears to be the serosa. No metastases are found in any of the internal organs."

These histologic findings are quoted because Forbus believes that it is quite easy to differentiate between these growths and adenocarcinoma on a morphologic basis without depending upon the complicated and rather time-consuming silver impregnation method.

The cells of these tumors react to silver impregnation, which indicates their origin from chromaffine cells of the intestinal tract. These cells are found in the crypts of Lieberkühn throughout the gastrointestinal tract. They are situated between the cylindrical cells of the mucosa, some extending from basement membrane to lumen, while others remain without contact with the lumen. Silver staining granules are always demonstrable in these cells and are always located between the basement membrane and the nucleus. The vacuoles in the cells are probably secondary to the formation of granules.

Careful histologic studies with the proper technic have demonstrated the origin of these tumors from the cells of Koltschitzky and Schmitt in the crypts of Lieberkühn. They are tumors of the chromaffine system, the granules of the cells having the ability to reduce an ammoniacal solution of silver.

The clinical peculiarities and the rather constant percentage of occurrence in the different series of appendices removed at operation are explained by this new conception of the origin of these tumors. The term, argentaffine tumor, should be adopted for this group of tumors, as they are not malignant, even in those cases in which the cells have infiltrated to the serosa or into the fat of the mesenteriolum. A careful histologic study has led to the recognition of the character of this peculiar group of growths and has explained their rather constant percentage of occurrence and peculiarities in clinical course notwithstanding that in many cases they have decidedly invasive characteristics.

—D. L.

A Resume of the Scarlet Fever Situation

THE recent work of the Dicks has naturally been followed by a renewed interest in and generalized study of scarlet fever, its etiology, treatment, and prophylaxis.

Park,* in a recent paper, ably reviews the past and present work concerning this disease and outlines its present status in relation to the newer developments.

Because of its comprehensive character and the information it contains, the communication is here summarized.

Among the earliest to attempt to produce a curative serum was Moser, in 1902, who immunized horses with streptococci obtained from toxic cases of scarlet fever.

Savchemko, in 1905, demonstrated that the broth in which such cultures were grown contained a strong toxin and that, therefore, it should also be utilized in the preparation of curative serum. He demonstrated, too, the presence of antitoxic as well as streptococcidal bodies in the serum thus produced.

Gabritschewsky, in 1907, advocated the use of a vaccine containing both streptococci and the broth in which they were grown, and used such a vaccine in human beings with varying success in prophylaxis.

Encouraging reports were made of this product in 1921 by Poletevkova.

The work of the Dicks proved, for the first time, the etiologic relation of streptococci to scarlet fever and led to the development of their skin test for susceptibility and to the evolution of methods for the effective active prophylactic immunization of exposed individuals.

Through the work of the Dicks and Dochez, a scarlet fever antitoxin has also been produced, the results of the use of which are reported by Park.

After intravenous administration, very striking symptomatic results have been observed, and its early use very probably prevents the development of complications. After the rash has disappeared it is useless and has no effect on the later septic complications.

The effect on mortality has not as yet been striking, but it may be expected to become evident as the use of the serum extends and, especially, when its administration is early and in sufficient amount.

The adoption of a refined serum and a unit dose is strongly advocated. One unit is suggested as the quantity of antitoxin required to neutralize one hundred skin test (Dick) doses. The addition of a serum containing streptococcidal, as well as antitoxic, bodies, as advocated by Moser, merits careful investigation and is worthy of trial, as it is very likely to increase curative efficiency.

—*R. A. K.*

*Park, W. H.: Scarlet Fever: Etiology, Prevention by Immunization, and Antitoxic Treatment, Jour. Am. Med. Assn., Oct. 17, 1925, lxxxv, 1180.

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The Annual Meeting

The 1926 Convention of the American Society of Clinical Pathologists will be held in Dallas, Texas, April 15, 16, and 17, 1926.

Elaborate preparations are being made by our Texas members to give their colleagues a truly southern hospitable reception. Entertainment will be provided for the visitors and their wives. Drs. Moursund and Black have already called a conference of all the clinical pathologists of Texas at which plans were laid and arrangements perfected to make this meeting a memorable one in the annals of our organization.

Make Your Hotel Reservations Now!

In view of the great influx of physicians in Dallas for the American Medical Association meeting, hotel accommodations will necessarily be at a premium. Inasmuch as the A. S. C. P. meets during the week preceding the A. M. A. convention, there will be room for all our members during our own meeting, but unless reservations are made right now, the rooms cannot be held for those who desire to stay over the ensuing week.

The Baker Hotel has been reserved as headquarters for the A. S. C. P. This hotel offers ideal meeting rooms for our purpose.

All Clinical Pathologists Are Invited

It has become a tradition with the A. S. C. P. to invite all clinical pathologists to our annual conventions be they members or not. To this end about five hundred invitations have been sent out to nonmembers extending to them the facilities of the Society.